THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL OF
THE AMERICAN TRUDEAU SOCIETY

EDITOR
MAX PINNER, New York, N. Y.

EDITORIAL BOARD

JOHN ALEXANDER, Ann Arbor, Mich.
J. BURNS AMBERSON, JR., New York, N. Y.
E. R. BALDWIN, Saranac Lake, N. Y.
H. J. CORPER, Denver, Col.
F. S. DOLLEY, Los Angeles, Calif.
BRUCE H. DOUGLAS, Detroit, Mich.
L. U. GARDNER, Saranac Lake, N. Y.
ROSS GOLDEN, New York, N. Y.
ESMOND R. LONG, Philadelphia, Pa.
LEWIS J. MOORMAN, Oklahoma City, Okla.
D. W. RICHARDS, JR., New York, N. Y.

VOLUME L
JULY-DECEMBER, 1944

PUBLISHED MONTHLY
AT MT. ROYAL AND GUILFORD AVENUES, BALTIMORE 2, MD.
BY THE NATIONAL TUBERCULOSIS ASSOCIATION

Medical
Nahr

CONTENTS: ORIGINAL ARTICLES

Number 1, July, 1944

A Comparison of Roentgenograms with the Pathology of Experimental Miliary Pulmonary Tuberculosis in the Rabbit. E. M. Medlar, G. S. Pesquera and W. H. Ordway 1

Patients Discharged Alive from a County Sanatorium. D. R. Hastings and Borghild Behn 24

Tuberculoma of the Posterior Mediastinum. Brian Blades and David J. Dugan 41

Loeffler's Syndrome. H. B. Pirkle and Julia R. Davin 48

Chemotherapy of Clinical Tuberculosis with Promin. H. Corwin Hinshaw, Karl H. Pfuetze and William H. Feldman 52

Cultivation of the Bovine Tubercle Bacillus. Andres R. Arena and Abel Cetrangolo 58

The Cutaneous Activity of Old Tuberculins Prepared by the Same and by Different Methods. R. Y. Gottschall, A. B. Mitchell and C. J. Stringer 68

Further Observations on the Production of Autolytic Tuberculin. H. J. Corper and Maurice L. Cohn 81

Number 2, August, 1944

Syphilis and Pulmonary Tuberculosis in the Negro. Reuben Hoffman and George G. Adams 85

Nutrition in Tuberculosis as Evaluated by Blood Analysis. Horace R. Getz, Irene S. Westfall and Howard J. Henderson 96

Epidemiology of Reinoculation Tuberculosis. F. M. Pottenger 112

Tuberculous Pneumonia. D. O. Shields 122

Recent Advances in the Campaign against Tuberculosis. R. G. Ferguson 131

Compulsory Hospitalization of Open Cases of Tuberculosis. Andrew L. Banyai and Anthony V. Cadden 136

The Tuberculosis Rejectee. Emil Bunta 147

Mediastinal Herniation in Artificial Pneumothorax. I. D. Bobrowitz 150

Effect of Promin on Experimental Tuberculosis. A. R. Armstrong, M. V. Rae, C. C. Lucas and P. H. Greey 160

Sulfanilamide and Sulfapyridine in Experimental Tuberculosis. C. Richard Smith 163

The Bactericidal Action of Stilboestrol on Tubercle Bacilli. G. H. Faulkner 167

Brief Reports:

Tracheotomy for Tuberculous Laryngeal Stenosis. Emil Rothstein and H. B. Pirkle 176

Pleural Changes in Oleothorax. Peter A. Herbut and Robert Charr 179

American Trudeau Society:
Officers, Executive Committee, Council Members and Advisory Board, 1944–1945 182
Honorary Members 183
Deaths of Members, 1943 184

NUMBER 3, SEPTEMBER, 1944

The Evolution of the Rest Treatment of Pulmonary Tuberculosis. JOSEPH H. PRATT 185
Evaluation of Chemotherapeutic Agents in Clinical Tuberculosis. H. CORWIN HINSHAW AND WILLIAM H. FELDMAN 202
Mass Chest Roentgenography and Admissions to Olive View Sanatorium. JOSEPH GOORWITCH 214
Basal Metabolism in Pulmonary Tuberculosis. GEORGE C. LEINER 223
Nutritional Studies in Tuberculosis. III. Thiamine (Vitamin B_1) Deficiency and Peripheral Neuritis. JASON E. FARBER AND D. K. MILLER 229
Cardiorespiratory Testing in Tuberculosis. K. ALBERT HARDEN, HOWARD M. PAYNE, MAURINE P. WEAVER AND JAMES R. LAUREY 234
To-morrow's Tuberculosis Associations. WILLIAM P. SHEPARD 244
Perspectives and Trends in Tuberculosis. HENRY STUART WILLIS 251
Editorial—Evaluation of Chemotherapy. MAX PINNER 257
Obituary—H. Kennon Dunham, 1872—1944 260
American Trudeau Society:
Report of the Program Committee 262
Report of the Committee on Therapy 263
Report of the Committee on Clinic Procedure 264
Report of the Committee on Medical Information 266

NUMBER 4, OCTOBER, 1944

Spirometric and Bronchospirometric Studies in Pneumothorax. GEORGE C. LEINER 267
Bronchography in Pulmonary Tuberculosis. I. Normal or Questionable Roentgenographic Findings in Lungs and Positive Sputum. B. A. DORMER, J. FRIEDLANDER AND F. J. WILES 283
Bronchography in Pulmonary Tuberculosis. II. Radiographic Black-out —Evaluation of Underlying Lesions. B. A. DORMER, J. FRIEDLANDER AND F. J. WILES 287
Injuries of the Thorax in Battle Casualties. L. KRAEER FERGUSON AND ROBERT B. BROWN 293
Round Densities within Cavities. I. D. BOBROWITZ 305
Tuberculosis of the Antrum. DAVID B. RADNER AND FORREST J. PINKERTON 313
The Fate of Very Young Children with Tuberculosis. GERTRUDE F. MITCHELL AND HENRY STUART WILLIS 316

Tubercle Bacilli in the Stomach Contents of Healthy, Normal Adults Exposed to Tuberculosis. C. Richard Smith 330
Specific Cytotoxic Action of Tuberculin. Dorothy H. Heilman, William H. Feldman and Frank C. Mann 344
Oxygen Lavage of the Pleural Space. James F. B. Zweighaft 357
American Trudeau Society:
Report of the Membership Committee 359
Report of the Committee on X-ray Apparatus and Technique 360
Report of the Committee on Tuberculosis in Industry 362
Report of the Medical Advisory Committee on Health Education 364

Number 5, November, 1944

Outlook for Tuberculosis Control in the Civilian Population. Thomas Parran 365
The Problem of the Tuberculous Veteran. Louis I. Dublin 375
The Tuberculosis Program of the Veterans Administration. Roy A. Wolford 380
Program for Tuberculous Patients among World War II Veterans. T. O. Kraabel 391
Tuberculosis and War. Summary. Esmond R. Long 401
The Indispensability of Routine X-ray Examinations of the Chest in a General Clinic. Robert G. Bloch and William B. Tucker 405
Promizole in Tuberculosis. William H. Feldman, H. Corwin Hinshaw and Frank C. Mann 418
Books 441
American Trudeau Society:
Report of the Committee on Standard Laboratory Procedure 465
Report of the Committee on Coexistent Syphilis and Tuberculosis 469
Report of the Committee on Coöperation with the American Board of Internal Medicine 470
The First National Mexican Congress on Tuberculosis and Silicosis. Mexico, D. F.—July 23 to 29, 1944 471

Number 6, December, 1944

The Effect of Sensitivity on the Distribution of Tubercle Bacilli in Tuberculosis. William L. Brosius and C. Eugene Woodruff 473
The Significance of Tubercle Bacilli in Gastric Contents. David D. Feld 481
The Demonstration of Tubercle Bacilli in an Employee Group with Clinically Inactive Pulmonary Tuberculosis. Edgar M. Medlar and Ada Chree Reid 490
Closure of Tuberculous Cavities. John Loesch 500
Bovine Tuberculosis in the United States. H. R. Smith 520
Specific Metabolic Products of the Tubercle Bacillus. John T. Riordan 534
Promin Inhalation Therapy in Pulmonary Tuberculosis. James S. Edlin, I. D. Bobrowitz, F. K. Safford, Jr. and Frank S. Butler 543

Statistical Data. What is Happening to the Tuberculosis Death Rate? ... 556
Obituary—Edward S. McSweeny, 1877–1944 ... 569
American Trudeau Society:
Report of the Committee on Undergraduate Medical Education ... 571
Some Suggested Procedures for the Early Period of Therapeutic Pneumothorax.—A Report of the Committee on Therapy ... 573
Report of the Michigan-Wisconsin-Minnesota Regional Therapy Conference ... 575

JUL 18 1944

THIS ISSUE CONTAINS ABSTRACTS

Vol. L JULY, 1944 No. 1

National Library

THE AMERICAN REVIEW OF TUBERCULOSIS

OFFICIAL JOURNAL
AMERICAN TRUDEAU SOCIETY

EDITOR
MAX PINNER, New York, 67, N. Y.

EDITORIAL BOARD

JOHN ALEXANDER, Ann Arbor, Mich.
J. BURNS AMBERSON, JR., New York, N. Y.
E. R. BALDWIN, Saranac Lake, N. Y.
H. J. CORPER, Denver, Col.
F. S. DOLLEY, Los Angeles, Calif.
BRUCE H. DOUGLAS, Detroit, Mich.
L. U. GARDNER, Saranac Lake, N. Y.
ROSS GOLDEN, New York, N. Y.
ESMOND R. LONG, Philadelphia, Pa.
LEWIS J. MOORMAN, Oklahoma City, Okla.
D. W. RICHARDS, JR., New York, N. Y.

PUBLISHED MONTHLY
at Mount Royal and Guilford Avenues, Baltimore 2, Md.
by the
National Tuberculosis Association
Business Office, 1790 Broadway, New York 19, N. Y.

Copyright 1944, by the National Tuberculosis Association

CONTENTS

E. M. Medlar, G. S. Pesquera and W. H. Ordway. A Comparison of Roentgenograms with the Pathology of Experimental Miliary Pulmonary Tuberculosis in the Rabbit.. 1

D. R. Hastings and Borghild Behn. Patients Discharged Alive from a County Sanatorium..... 24

Brian Blades and David J. Dugan. Tuberculoma of the Posterior Mediastinum..... 41

H. B. Pirkle and Julia R. Davin. Loeffler's Syndrome. Transient Pulmonary Infiltrations with Blood Eosinophilia..... 48

H. Corwin Hinshaw, Karl H. Pfuetze and William H. Feldman. Chemotherapy of Clinical Tuberculosis with Promin. P,P′-Diaminodiphenylsulfone-N, N′-Didextrose Sulfonate. A Second Report of Progress..... 52

Andres R. Arena and Abel Cetrangolo. Cultivation of the Bovine Tubercle Bacillus..... 58

R. Y. Gottschall, A. B. Mitchell and C. J. Stringer. The Cutaneous Activity of old Tuberculins Prepared by the Same and by Different Methods..... 68

H. J. Corper and Maurice L. Cohn. Further Observations on the Production of Autolytic Tuberclin..... 81

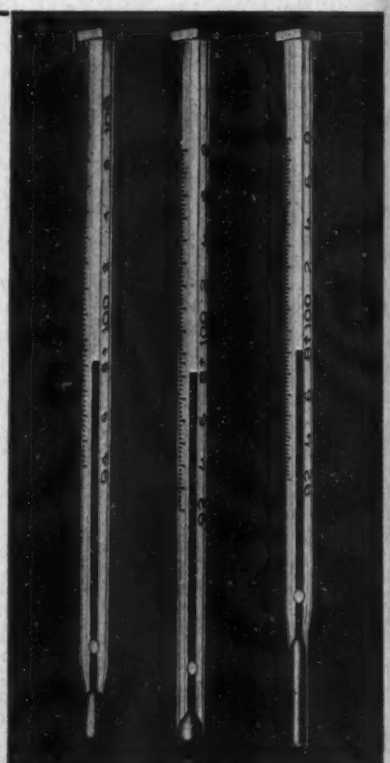
Use **FAICHNEY**
"Tempglass"
THERMOMETERS
for Clinical Efficiency and Greater Economy....

These fine thermometers are *easier to read* because calibrations are spaced 25% farther apart than on ordinary clinical thermometers. In addition, every Faichney Thermometer is *shake-tested* for quick, easy shake-down. These are points that contribute to clinical efficiency and time-saving.

The Faichney "Tempglass" process of tempering glass means less frequent replacement of thermometers and consequently *greater economy*... because there is less chance of breakage. "Tempglass" thermometers are *tough* and *sturdy*—and the triangular top prevents "rolling" casualties.

Designed especially for sanatorium use, Faichney Tempglass clinical Thermometers will meet your most exacting requirements. Standardize with "Faichney's". Your choice of any style bulb... at pre-war prices.

FAICHNEY INSTRUMENT CORP.
WATERTOWN, NEW YORK
Manufacturers of Thermometers Since 1888



Entered as second-class matter March 9, 1917, at the post-office at Baltimore, Md., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in section 1103, act of October 3, 1917. Authorised on July 2, 1918.

A COMPARISON OF ROENTGENOGRAMS WITH THE PATHOLOGY OF EXPERIMENTAL MILIARY PULMONARY TUBERCULOSIS IN THE RABBIT[1]

E. M. MEDLAR, G. S. PESQUERA AND W. H. ORDWAY

It is recognized that thoracic roentgenograms are indispensable in the study of pulmonary tuberculosis in man, especially in the minimal stage of the disease and in demonstrating instability of lesions. Whenever any method of examination becomes indispensable it is important that its limitations be understood. The bony framework and the denser organic tissues of the thorax, the size and density of the tuberculous foci and the pathological interpretations of shadows observed in roentgenograms of the pulmonary fields are important factors that need scrutiny. The main concern to date has been to develop the apparatus and the technique to yield comparable roentgenograms of the lung fields and detail therein. Interference by the denser thoracic structures has been circumvented to some degree by the use of stereo-roentgenograms; oblique, lateral and lordotic positions; spot films; the fluoroscope and planigraphy.

That tuberculous foci may be present in the pulmonary parenchyma and not be visualized in roentgenograms of the thorax is not too generally appreciated. Demonstration of small tuberculous cavities at necropsy when they are not seen in roentgenograms of the chest during life is not unusual. However, the authors have been unable to find reports in the literature of studies on man which have compared thoracic roentgenograms with those of excised and inflated lungs and with the actual pathological findings. Dunham and Skavlem (1) made a roentgenological study of excised and inflated lungs from individuals who succumbed to tuberculosis and found that "certain densities suggest definite cellular pathology" and "etiology is deduced by a study of the entire lung fields." The greatest value was obtained when roentgenograms were interpreted in terms of definite pathology but "almost any pathology can be read into a plate by a clinician with a keen imagination." The need for a standard terminology and a standard interpretation of roentgenograms was fully appreciated by these authors.

Opie and Andersen (2) have shown that roentgenograms of lungs, excised and inflated, from persons who died from causes other than tuberculosis reveal calcific remnants of tuberculous infection in over 90 per cent of the specimens they examined. In a later publication Opie (3) states: "Changes produced in lungs are defined with much more exactness by X-ray films of the excised organ and definition of lesions is clearest if the lungs are first inflated." Miller (4) found calcified foci in 69 per cent of 273 pairs of lungs obtained from individuals over fourteen years of age who died from causes other than tuberculosis and in 24 per cent of 1,220 patients in a diagnostic series. No comparative study of antemortem roentgenograms of the thorax and of the excised lungs was mentioned. These

[1] From the Hegeman Memorial Laboratory and Metropolitan Life Insurance Company Sanatorium, Mount McGregor, New York.

two reports deal with material obtained from large urban centres and are concerned primarily with the problem of tuberculous infection in such areas. The calcified foci have been interpreted as remnants of primary infection. The following quotations from the article by Miller reveal some of the limitations of roentgenography in studies of human lungs. "In at least 23 per cent of adults the total calcification in these lesions is so minute that it only rarely casts a characteristic shadow on the roentgenograms of the excised lungs." "With a painstaking technique and accurate diagnostic criteria, calcification of pulmonary first infection in the living individual is recognizable in about one-fourth of all cases in which it exists, while in individuals with an appreciable extent of calcification it is demonstrated in about one-third of the cases." "Less than one-fifth of the total number of calcified lesions of appreciable extent are demonstrated in living subjects."

The interpretation of the nature of tuberculous lesions from the types of shadow observed in chest roentgenograms depends in large degree upon inference since it is not possible to compare, in most instances, the film with the pathological changes present at the time the roentgenogram was taken. This problem, which has been investigated to some extent, is to be discussed in this paper. Miller states: "Solitary fibrous or fibro-caseous nodules in the lung parenchyma may cast a shadow simulating a partially calcified focus" and "absorption of roentgen rays varies directly with the total density of the exposed substance." Steiner (5) attempted to correlate the histopathology found postmortem with antemortem roentgenograms of the chest in cases of miliary pulmonary tuberculosis. Unfortunately no films of the excised lungs were made in this study. He found that in about half of the cases the roentgenograms of the thorax had been interpreted as negative for tuberculosis. There was a closer correlation between the roentgenograms and the histopathology than between either size or number of lesions and there was a lesser correlation with size than with numbers. "Epithelioid" tubercles, even of considerable size and number, failed to cast shadows, whereas shadows were evident if collagen fibrils or caseous material was present, although the shadow was not due solely to the caseous substance. None of the tubercles contained significant amounts of calcium and incineration failed to reveal an appreciable difference in the ash content of the different types of lesions.

Roentgenographic studies of pulmonary tuberculosis produced experimentally in animals have been reported. Large numbers of tubercle bacilli have been introduced into the lungs by aspiration, by injection into the trachea through a syringe or into selected bronchi through a bronchoscope. By such methods a considerable area of tuberculous pneumonia was produced at the outset. Soper *et al.* (6) infected normal and vaccinated rabbits through aspiration of virulent bovine tubercle bacilli (0.5 to 5 mg.). No shadows were observed before sixteen days in the primary infection if the dosage was small but, if large, shadows were seen in a week. Reaction to reinfection was more intense and earlier. Shadows persisted, with fluctuations, in primary infection to the death of the animal. The initial pneumonic reaction could undergo almost complete resolution in both normal and vaccinated animals with the resolution being more rapid, more

complete and more constant in the vaccinated series. No roentgenograms of excised lungs were made and there was no attempt to correlate the roentgenogram shadows with the pathological changes at different periods during the study.

Burke (7) conducted a comparative study of roentgenograms of the thorax and of excised and inflated lungs with the pathological findings existing at the date the roentgenograms were taken. Normal and vaccinated rabbits were given an intratracheal injection of 1 mg. of virulent human type of tubercle bacilli. A localized tuberculous pneumonia was produced by keeping the animals in certain positions during and for a short time after the suspension of bacteria was introduced. Roentgenographic shadows were first observed in primary infection in six to eight days in the excised lungs and in ten to seventeen days in the thorax. Reaction to reinfection was so prompt that shadows were demonstrable in roentgenograms of both thorax and excised lungs in twenty-four hours. The character of the shadows failed to yield satisfactory information as to the nature of tuberculous lesions, for fibrous and fibrocaseous foci cast about the same shadows as primitive, poorly defined tubercles. Burke states: "The roentgenograms of the excised lungs, like the chest roentgenograms, failed in many instances to portray the nature of the disease" and "no shadow or combination of shadows was found in the chest roentgenograms or of the excised lungs which served to distinguish the lesions of reinfection experimental pulmonary tuberculosis from those of primary experimental pulmonary tuberculosis." Lesions not observed in roentgenograms of the thorax were often revealed in roentgenograms of the excised lungs.

Mills *et al.* (8) produced areas of tuberculous pneumonia in dogs by introducing from 0.5 to 10 mg. of virulent human type tubercle bacilli into selected bronchi through a bronchoscope. Roentgenograms of the thorax and of excised lungs comprised an important part of their studies. The results obtained were very irregular due to the inherent resistance of the dog to tuberculous infection. The ability to demonstrate the lesions seemed to depend upon the site of inoculation and upon the amount of tubercle bacilli introduced. Small cavities not seen in roentgenograms of the thorax were evident in films of excised lungs and small foci of calcium deposits seen in roentgenograms of excised lungs were not evident in films of the thorax.

These experimental studies do not present a critical analysis of the limitations of roentgenograms to portray tuberculous lesions, since a considerable area of tuberculous pneumonia was present at an early date. In man this condition appears subsequent to a considerable bronchogenic spread of the disease from a preëxisting focus. While the pathological nature of a large pneumonic tuberculous lesion differs only in volume from that of a minute bronchopneumonic focus, the importance given to the discovery of the disease in its minimal stage makes it necessary to determine the size, content and approximate age of small tubercles when first demonstrable in roentgen-ray films. It would seem that the best approach to this problem would be to investigate experimentally produced miliary pulmonary tuberculosis.

In previous reports one of us (9, 10, 11) has shown that miliary pulmonary

tuberculosis in the rabbit can be produced consistently by intravenous injection of bovine bacilli. The number of lesions can be controlled to a degree by the number of bacilli injected and foci are dispersed throughout the lung parenchyma with the initial seeding. The course of the infection depends upon whether virulent or nonvirulent bacilli are used, whether normal or vaccinated animals are selected for the inoculation of virulent bacilli and whether a large or small dosage of virulent bacilli is given.

In brief the pathogenesis of the nonvirulent disease is as follows: Microscopical lesions which consist of small focal areas of congestion of alveolar walls and a slight inflammatory reaction of neutrophils and monocytes are demonstrable at twenty-four hours. At one week, minute lesions are recognizable macroscopically after formaldehyde fixation of the lung. Microscopically one finds the alveoli contiguous to the initial alveolar wall injury loosely filled with monocytes and a few neutrophils and lymphocytes in the majority of foci, while neutrophils are numerous in an occasional lesion. At two weeks, the tubercles are visible in the fresh specimens because of a greater accumulation of the cells of inflammation. There is a continued progression of the lesions up to four to six weeks and at this time two types, which are usually of equal size, are present. The most common type consists of monocytes and lymphocytes fairly compact in the central area and of a looser arrangement in the periphery. The area involved represents half a dozen or more alveolar spaces in cross section. The other type is composed of a dense "core" of neutrophils with a collar arrangement of monocytes and lymphocytes. The monocytic type of lesion regresses fairly rapidly and in three to six months the only evidence that such lesions existed is the presence of a few delicate strands of scar tissue or a few lymphocytes in an alveolar wall.

The course of the foci with a neutrophil "core" may vary. The lesion may not enlarge significantly and if this happens the "core" dies, becoming a small area of caseation around which monocytes may accumulate to form a giant cell. The peripheral portion is infiltrated with monocytes and lymphocytes and fibrous tissue is gradually formed in and around the focus. Some of the lesions may attain a size of 3 mm. in diameter with a large caseous "core" which gives a yellowish tint to the tubercle. These larger foci differ from the smaller ones in that calcium may be deposited in the caseous areas and that they may persist for a year or more before complete organization is accomplished. The end-result may be a fibrous-walled area of calcification or a rather dense fibrous scar with a sparse infiltration of lymphocytes.

The pathogenesis of the virulent infection differs from the nonvirulent during the first week only in that neutrophils are more numerous in the lesions. At two weeks, when the foci have become visible, the alveoli adjacent to the original site of deposit of the tubercle bacilli have become filled with a mixture of neutrophils and monocytes with a nidus of closely packed neutrophils. A few of these small abscesses have already begun to erode and to discharge their content of pus and tubercle bacilli into bronchi or bronchioles. If two foci in the parenchyma are in close proximation they may have become confluent. Monocytic tubercles

are few in number and are usually of small size. At three weeks, the majority of the original parenchymal foci have increased considerably in size by involving the contiguous normal lung tissue and by confluence of adjacent lesions. Necrosis of the neutrophil "core" presents the picture of early caseation. Some tuberculous abscesses adjacent to bronchioles or bronchi have ruptured into the bronchial tree and bronchogenic spreads to previously normal lung parenchyma have occurred. This process continues to the death of the animal, with the large majority of parenchymal units eventually becoming involved. Survival time ranges from one to six months, dependent in large part upon the size of the infecting dose with variations in resistance of individual animals affecting the outcome only in prolonging the weeks of survival. Microscopical examination of the extensively involved lung reveals considerable areas of caseation, representative of original parenchymal foci and of old bronchogenic spreads; areas of tuberculous bronchopneumonia (recent bronchogenic spreads) in which the inflammatory exudate of neutrophils and monocytes are well preserved; small bronchi and bronchioles filled with tuberculous pus; and tuberculous abscesses which have opened into the bronchial tree. Very little evidence is found of increase of fibrous tissue, of calcification of the caseous foci or of giant cell formation—evidence of beginning repair of the foci.

If rabbits are vaccinated by an intravenous injection of nonvirulent bacilli six months or more prior to intravenous reinfection with virulent organisms the course of the disease varies considerably in a group of animals. The inflammatory response is more prompt in the vaccinated rabbits so that foci at one week ordinarily are as large as at the end of two weeks in the nonvaccinated animals. At this stage the alveolar capillaries in and around the lesions are considerably congested. In an occasional animal the disease steadily progresses to death and the macroscopical and microscopical lesions closely resemble those of a primary virulent infection. In the majority of instances a large proportion of the lesions, especially in the central and ventral portions of the lung parenchyma, begins to retrogress in about a month with more or less complete resolution having occurred within a six-month period. Foci in the caudal and dorsal portions of the parenchyma tend to progress slowly with the development of considerable areas of tuberculous bronchopneumonia from peripheral enlargement and local bronchogenic "spreads." Eventually these areas soften and, with discharge of the necrotic material, cavitation and bronchogenic "spreads" to distant portions of lung tissue may occur. This phase of the pathological process is essentially the same as that in nonvaccinated animals, except that the lesions are localized for a much longer time. If cavitation has not occurred at the end of several months it is not uncommon to find dense focal deposits of calcium salts in the caseated areas, a fibrous capsule of considerable extent in the periphery, and pleural adhesions over the involved area. Eventually all the animals die from tuberculous infection, a combination of renal and pulmonary disease deciding the issue.

With this knowledge of the pathogenesis of miliary pulmonary tuberculosis in the rabbit under a variety of conditions at our command an excellent oppor-

tunity is afforded to compare roentgenograms with the actual lesions in a series of experiments. This report will give, in general, the results obtained from such an investigation.

METHODS AND RESULTS

A group of 40 four-month old animals was given an intravenous inoculation of 10 mg. of nonvirulent living bovine tubercle bacilli (BCG). Six months later the vaccinated group and a group of 40 normal adult rabbits were given an intravenous injection of 0.005 mg. of virulent bovine tubercle bacilli. Another group of 40 normal adult animals was given an intravenous inoculation of 10 mg. of BCG.

The roentgenological study of these three groups was identical. Stereoscopic roentgenograms were obtained from each animal prior to the introduction of the bacilli, and at twenty-four, forty-eight and ninety-six hours and subsequently at weekly intervals after the bacilli were injected. Without reference to the roentgenograms, animals were sacrificed at one, two, three, four, seven, fourteen, twenty-one days, etc. The thoracic contents were removed; heart and great vessels, oesophagus and mediastinal tissues were carefully dissected away; and, with the lungs inflated to approximately normal intrathoracic size, stereoroentgenograms were obtained. The lungs were then preserved in 10 per cent formaldehyde for study of the pathological findings.

An animal board was constructed with stocks at one end to hold the forepaws and head firmly. Adjacent to the stocks and above the floor board a tunnel to accommodate a 5x7 inch cassette was fitted with two stops sufficiently far apart to allow the taking of stereo-roentgenograms. The top of the tunnel was covered with a sheet of heavy cellophane and on top of this was placed a sheet of lead, one-sixteenth of an inch thick, with an aperture of three and a half by five inches in the centre. Posterior to this the base was made level with the top of the lead sheet. An assistant controlled the animal in the proper position for roentgenography after the stocks had been applied.

The radiological technique used was as follows:

The X-ray tube focal-spot size was 1.5 mm. For roentgenograms of the thorax a target-film distance of 20 inches, a kilo-volt peak of 60, a milliamperage of 200 and an exposure of 1/120 of a second were found to give the best results when high speed screens were used. For roentgenograms of the excised and inflated lungs a target-film distance of 30 inches, a kilo-volt peak of 30, a milliamperage of 100 and an exposure of one-half second gave the best results with nonscreen film without an intervening filter. In taking roentgenograms of the excised lungs a cardboard exposure holder was so placed that the nonscreen film was in the same position as was the film in the cassette.

Since a generalized miliary pulmonary tuberculosis was being studied, the criterion for interpretation of roentgenograms of the thorax was that bilateral shadows, no matter how indistinct, had to be present before a positive reading was justifiable. The roentgenogram taken prior to the injection of the bacilli was used as the control for interpreting the serial films of each rabbit.

PRIMARY VIRULENT GROUP

When virulent bovine bacilli are introduced intravenously into a considerable group of rabbits the course of the infection is little altered by differences in natural resistance of individual animals, even if the dosage is small. The pathogenesis of the disease is so uniform that for purpose of illustration serial roentgenograms of the thorax of an animal surviving the longest time can be used to demonstrate the sequence of events. To illustrate the progression of the disease in roentgenograms of excised lungs, films may be used from animals sacrificed at dates represented in the serial roentgenograms of the thorax, for the various animals are comparable at any given date. This is the procedure used in figures 1 to 16.

In roentgenograms of the thorax the first definite, though faint, bilateral shadows were observed at three weeks after inoculation in all animals. As the disease progressed the "snow storm" effect developed rapidly with the intrapulmonary shadows becoming larger and more dense and with an inequality in density being noted.

The best way to appreciate the volume of lesions in roentgenograms of excised lungs is to observe how the arborizations of the vascular tree are obscured as the disease progresses. The vascular tree in figures 2 and 4 is comparable, while in figure 6 it is less distinct, especially in the more voluminous portion of the lungs. No individual lesions are in evidence in figure 6, although a considerable amount of disease is present. The vascular tree becomes more and more obscure in figures 8 to 16. The volume of lesions is much more evident in the excised inflated lung than in the roentgenograms of the thorax prior to sacrifice. When the peripheral and central portions of the lung field are compared there appears to be much more disease in the central areas, although per unit of volume there is no more disease in one portion of the lung than in another. The superimposition of foci in the more voluminous portion does several things which should be borne in mind in the interpretation of a roentgenogram. An impression is gained that the whole of the lung parenchyma is involved whereas there are many normal parenchymal units interspersed between the tuberculous lesions. The denser shadows are caused by the superimposition of lesions rather than by any difference in the nature of the foci and this superimposition renders the individualization of lesions extremely difficult. The "fuzzy" outline of lesions in the central part as compared to the more distinct outline of the foci in the thin periphery does not indicate that one lesion has more "collateral" inflammation than the other. Where the larger foci can be individualized it is evident that a considerable volume of lung parenchyma is involved—a result of peripheral enlargement, confluence of contiguous foci and local bronchial "spreads." These shadows represent an area of tuberculous bronchopneumonia. Differentiation between areas of dense pus and of caseation is not evident. Figure 16 is of particular interest because of the "honeycomb" appearance of a part of the lung field, the effect being produced by the presence of normal parenchymal units between tuberculous foci. The circular areas are really spherical and, while

suggesting cavitation, are emphysematous bullae caused by a partial obstruction to the egress of air due to tuberculous foci encroaching upon a bronchus.

As the roentgenograms of the excised lungs reveal more disease than do roentgenograms of the thorax so does the macroscopical study of the lungs reveal more disease than do the roentgenograms of the excised lungs. Microscopically still more lesions are found, since microscopical lesions are demonstrable twenty-four hours after injection. From the initial reaction to the death of the animal the foci are mainly a progressive accumulation of leucocytes, neutrophils and monocytes in large part, with very little evidence of retrogression or of fibrosis. Giant cells are infrequently observed and then only in the late phase of a rare, small healing focus. Typical caseation first appears in about four weeks and is present in the areas of tuberculous pus which has failed to dissolve the necrotic tissue and be discharged. No lesion has a clean-cut periphery where the inflammatory exudate ceases and normal lung tissue begins. No evidence of calcium deposit was observed in any of the lesions. When a comparison is made of the microscopical appearance with the shadows in the roentgenograms of the excised lungs four facts are apparent. First, shadows of individual lesions are not cast in roentgenograms of excised lungs until a compact inflammatory exudate equal in volume to at least a primary lobule has occurred. Second, the loose "collateral" inflammation is not of sufficient density to cast a shadow so that the true border of a tuberculous focus is not visualized. Third, no differentiation between compact tuberculous pus and caseation is shown. And last, a considerable amount of inflammatory exudate is present before any shadows are cast.

DESCRIPTION OF PLATES

Figures 1 to 16 illustrate the primary virulent infection, Figures 17 to 30 the primary nonvirulent infection and figures 31 to 48 the reinfection group. Figures 1 to 15, odd numbers, are the serial roentgenograms of the thorax of one animal and in all other illustrations each chest roentgenogram represents an animal. Figures 2 to 14, even numbers, are roentgenograms of excised lungs from rabbits sacrificed on the same date as the companion roentgenogram of the chest in the serial set and in all other instances the roentgenograms of excised lungs and of the thorax are from the same animal. All roentgenograms of the excised lung were obtained within half an hour after the companion roentgenogram of the thorax. The labels, one week, etc., indicate the lapse of time after tubercle bacilli had been injected intravenously. The roentgenograms are practically self-explanatory and minute description of each illustration seems unnecessary. The most striking features of the roentgenograms are discussed in the text. The most important thing to bear in mind is that the differences in density present in the roentgenograms do not necessarily represent differences in pathological structure within tuberculous foci. Nontuberculous disease is shown in 2 instances—figures 19 and 20 where an acute lobar pneumonia is present and figures 29 and 30 where a nontuberculous infarct is present in the caudal portion of the lungs. The only illustrations in which considerable deposits of calcium were found are figures 47 and 48 and in this specimen the extent and amount of calcium deposit appeared to be greater on macroscopical and microscopical examinations than is evidenced by the small, dense dots and dashes in the roentgenograms. The circular areas in figures 2, 6 and 30 are caused by a large bubble of air between the lung and the paper on which it rested. The "softness" of the technique used in taking roentgenograms of excised lungs is shown by the shadow cast by ordinary adhesive tape, pieces of which are seen at the bottom of several of the pictures.

PLATE I

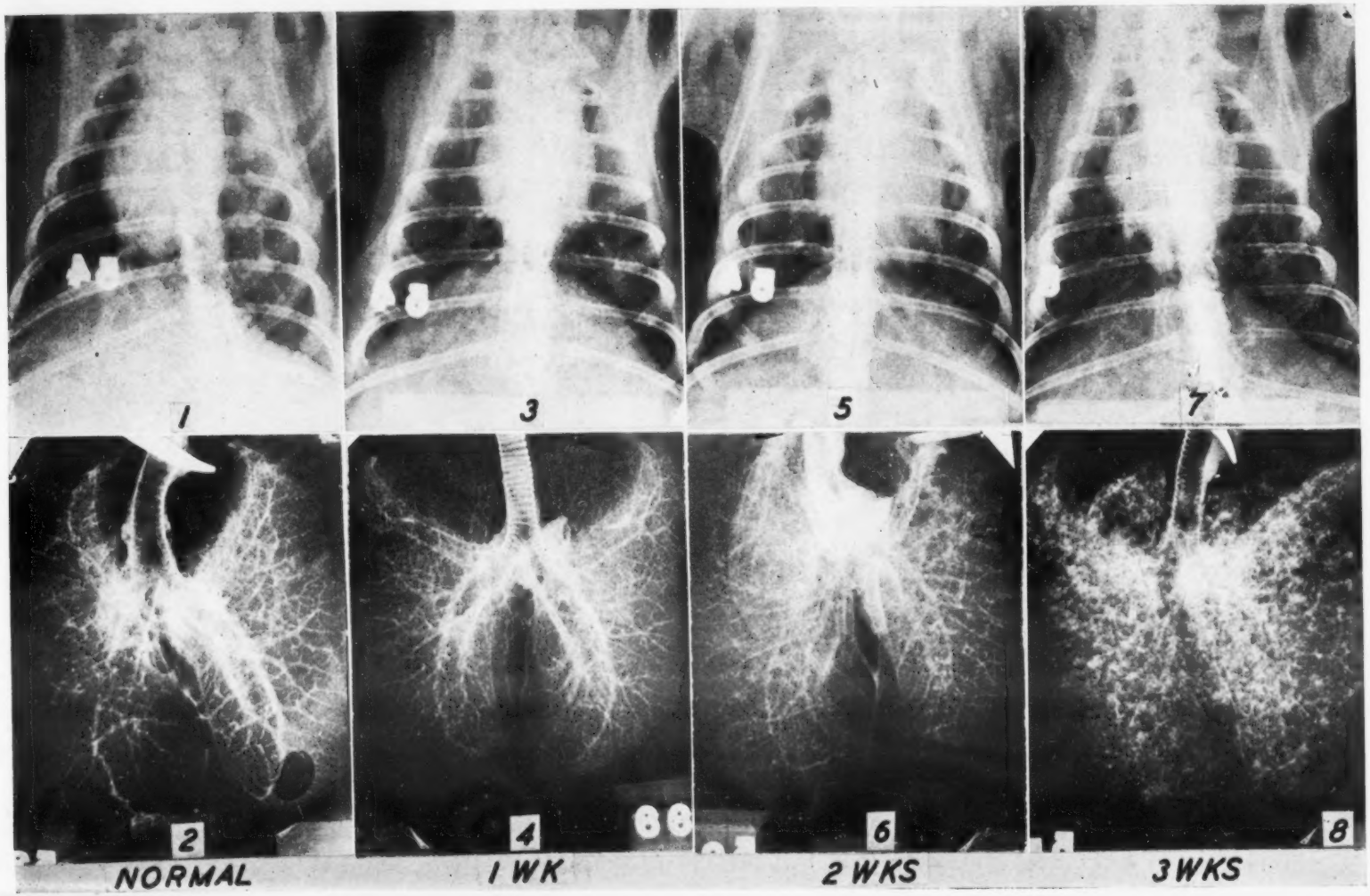


PLATE I

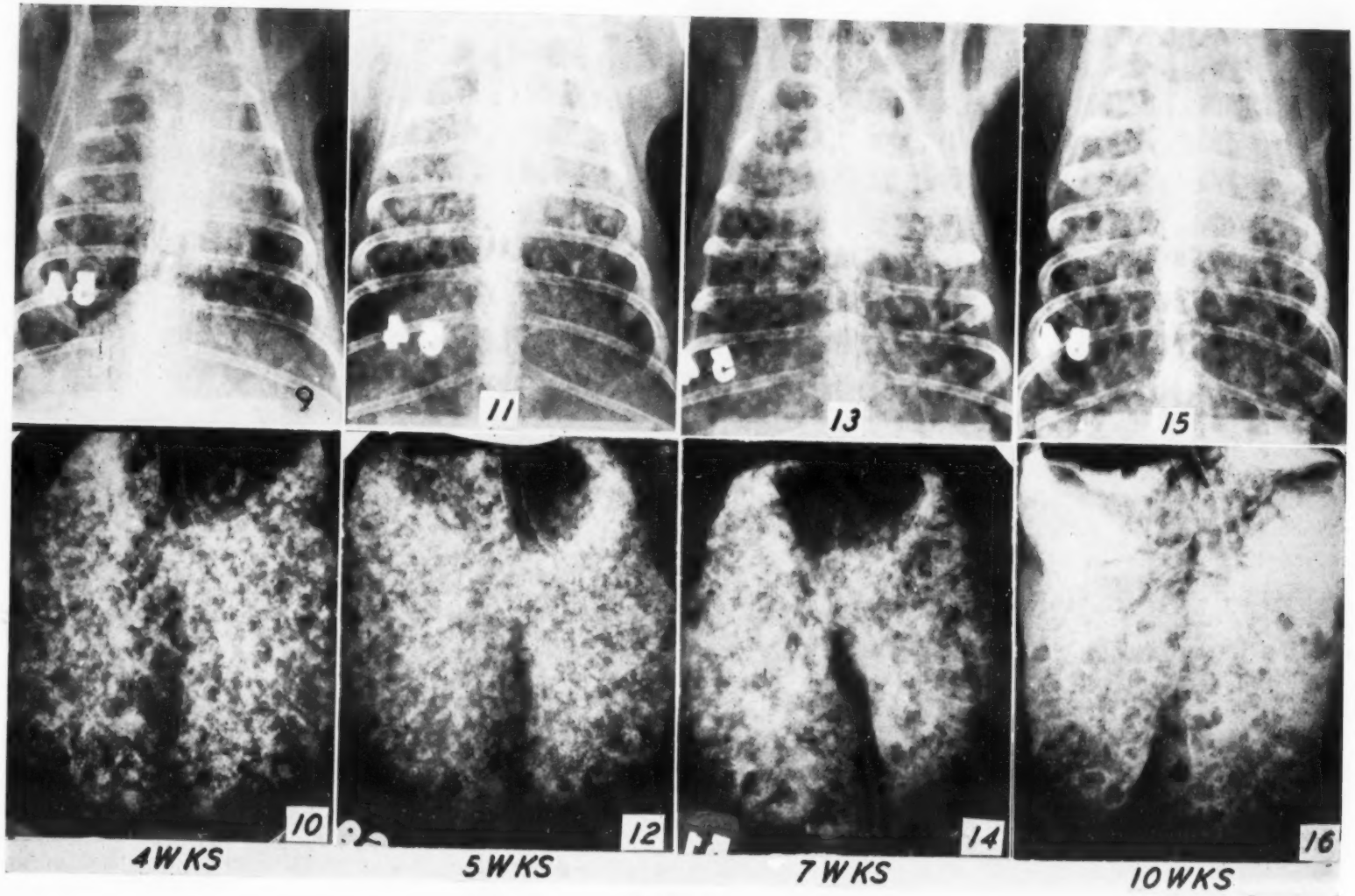


PLATE II

PRIMARY NONVIRULENT GROUP

In this group 10 mg. of BCG were injected intravenously, since previous studies had shown that in most instances the disease was of a mild character and that with this large dosage a very large number of tuberculous foci are produced. The size of the group allows for the demonstration of differences in individual resistance for there is a considerable variation in the amount of inflammatory reaction, the types of tuberculous foci and the rapidity with which the pulmonary foci are resolved. It is not possible to illustrate the pathological course as was done in the primary virulent group. Instead, individual animals have been selected to demonstrate the different phases of the disease as revealed in the roentgenograms and these are illustrated in figures 17 to 30.

In so far as roentgenograms of the chest are concerned the greatest amount of disease observed is shown in figure 23, where a rather faint "snow storm" effect is in evidence. A few of the animals showed no definite evidence of pulmonary involvement. Two illustrations in this series are of particular interest. Figure 19 shows a slight "haziness" in the upper lung fields where a nontuberculous pneumonia is present. This lesion can best be appreciated by comparing figures 19 and 21, the roentgenograms being taken on the same day. Figure 29 is from an animal six months after having received 10 mg. of BCG. Note that the shadows of the tuberculous lesions are more distinct and discrete in this roentgenogram than in figure 23, although they are still of the "soft" type of lesion. This animal had the same extent and type of shadow at one month as is shown in figure 23 and intervening roentgenograms were interpreted as "stationary."

The roentgenograms of the excised lungs represent the same animals as those in the roentgenograms which show the thorax and were taken within half an hour after the films of the thorax. As in the primary virulent infection, the amount of disease can best be appreciated by the sharpness in the arborization of the vascular tree. Much more disease is portrayed by these films than would be suspected from the chest roentgenograms. It is doubtful that any individual tuberculous lesions are shown in this series, except in figure 30. Where it seems that individual foci are evident the effect is caused by the superimposition of lesions, for the lungs contained so many small foci that it is impossible to avoid superimposition of many lesions, except at the thin periphery where no shadows are seen. The nontuberculous pneumonic consolidation and scattered patches of bronchopneumonia are of greater extent in figure 20 than one would suspect from figure 19. Note how the air-containing main arborizations of the bronchial tree are silhouetted within the consolidated areas. The indistinctness of the vascular tree in figure 20 away from the pneumonic consolidation suggests the presence of tuberculous lesions, although no individual foci are discernible. Figure 22 shows considerably more tuberculous disease than figure 20, although the animals were sacrificed on the same day after they had been inoculated—an example of individual variation. Figure 30 is of interest because it shows a bilateral nontuberculous infarct at the caudal tips, subsequent to infected thrombi in pulmonary vessels, which is not evident in the roentgenogram of the thorax as the lesions were located behind the diaphragm, and because of the

PLATE III

PLATE IV

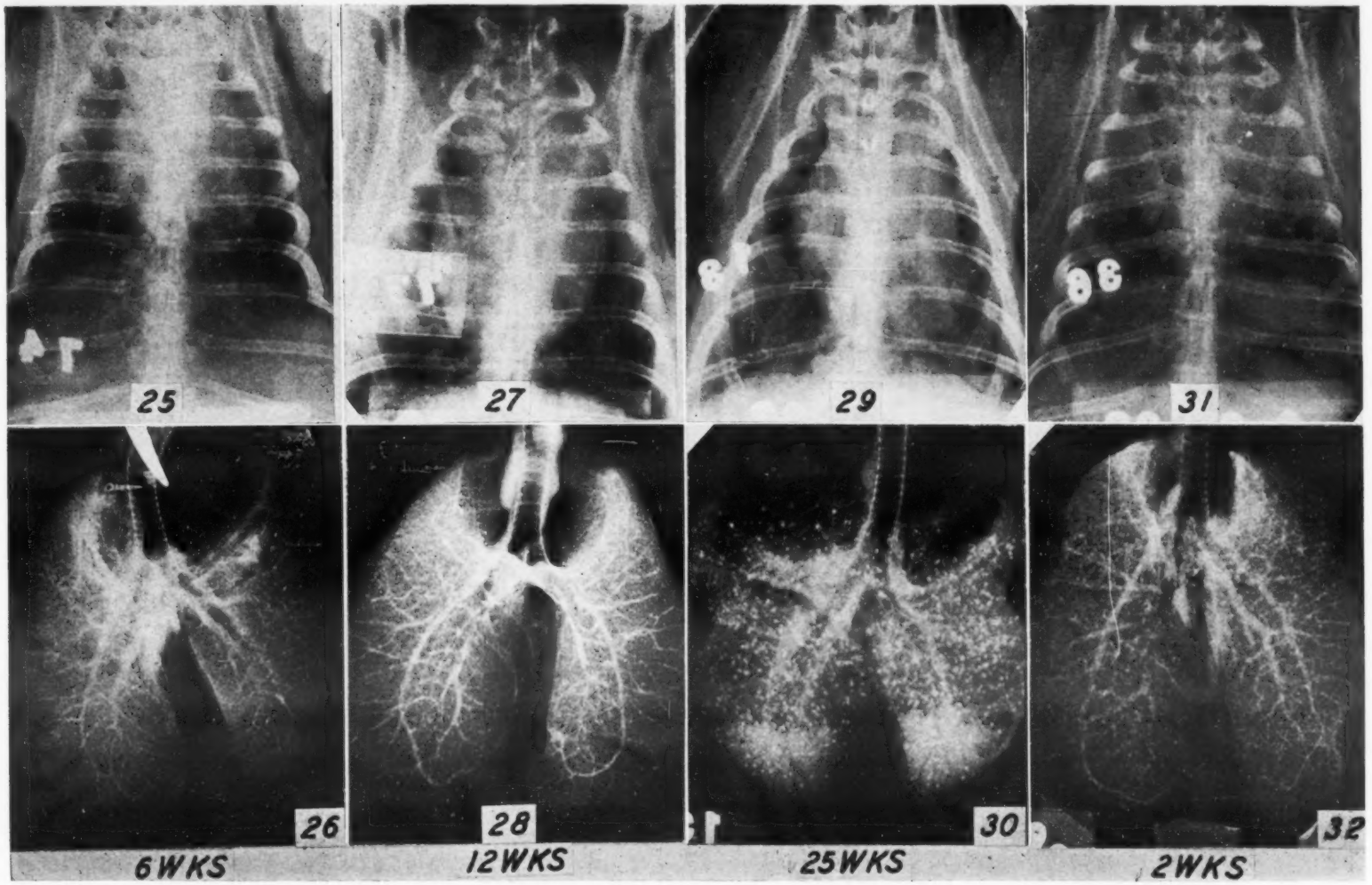


PLATE IV

PLATE III

considerable amount of tuberculous changes. The discrete dense tubercles will be discussed below in relation to the histopathology.

As with the primary virulent infection more disease was seen with the naked eye and still more was found microscopically than was observed in the roentgenograms. In the large majority the foci consisted histopathologically of a loose-meshed accumulation of monocytes with lymphocytes predominating in the late healing phase. A few of the lesions showed a densely packed "core" of neutrophils, of variable size, which later became the site of caseation. The size and final microscopical picture of these lesions varied considerably as time passed. Some showed "text-book" pictures of "tubercle" with increasing fibrosis as healing progressed. Some were composed largely of a caseous area with a thin fibrous capsule infiltrated chiefly with lymphocytes and on rare occasion considerable calcium might be present, at six months, in the periphery of the caseous portion. Except in the walled-off old lesions there was no sharp demarcation between the tuberculous focus and the surrounding normal lung tissue. The type of pathological finding in the older lesions is briefly described in the pathogenesis of nonvirulent infection earlier in this article and is illustrated (figures 3 to 13) in a previous article (10).

A comparison between the histopathological appearance and the roentgenograms suggests that the only tuberculous foci which, of themselves, can cast a shadow are those in which dense inflammatory exudate, caseation, fibrosis, calcification or a combination of these factors are present. Even when these factors are present, roentgenograms of the excised lung fail to differentiate between dense pus and caseation, fibrosis and caseation or caseation and calcification. All of the various factors are present in a variety of combinations in the lungs shown in figures 29 and 30 and an attempt to interpret the nature of the lesions in any of the dense tubercles from the roentgenogram shadows proved futile.

REINFECTED GROUP

This group of animals received a primary intravenous injection of 10 mg. of BCG, and six months later a reinfection dose of 0.005 mg. of virulent bovine bacilli was given on the same date as in the primary virulent group. From previous studies the irregular results obtained in roentgenograms of the thorax and of the excised lungs were anticipated. Roentgenograms of the thorax taken one month after the vaccinating dose revealed a fine "snow storm" effect in most of the animals and those taken just previous to the reinfection were interpreted as normal in all but one animal, figure 47. The irregular development of the disease after reinfection makes it necessary to select animals to demonstrate the variations observed and in each instance use is made of a roentgenogram of the thorax and of the excised lung taken within half an hour after the thoracic film. Figures 31 to 48 illustrate the pathological process as seen in the roentgenograms. None of the animals in this series died during the period of study.

The first definite evidence of shadows in the roentgenograms of the thorax was observed at three weeks, figure 33. Subsequently, there was a great varia-

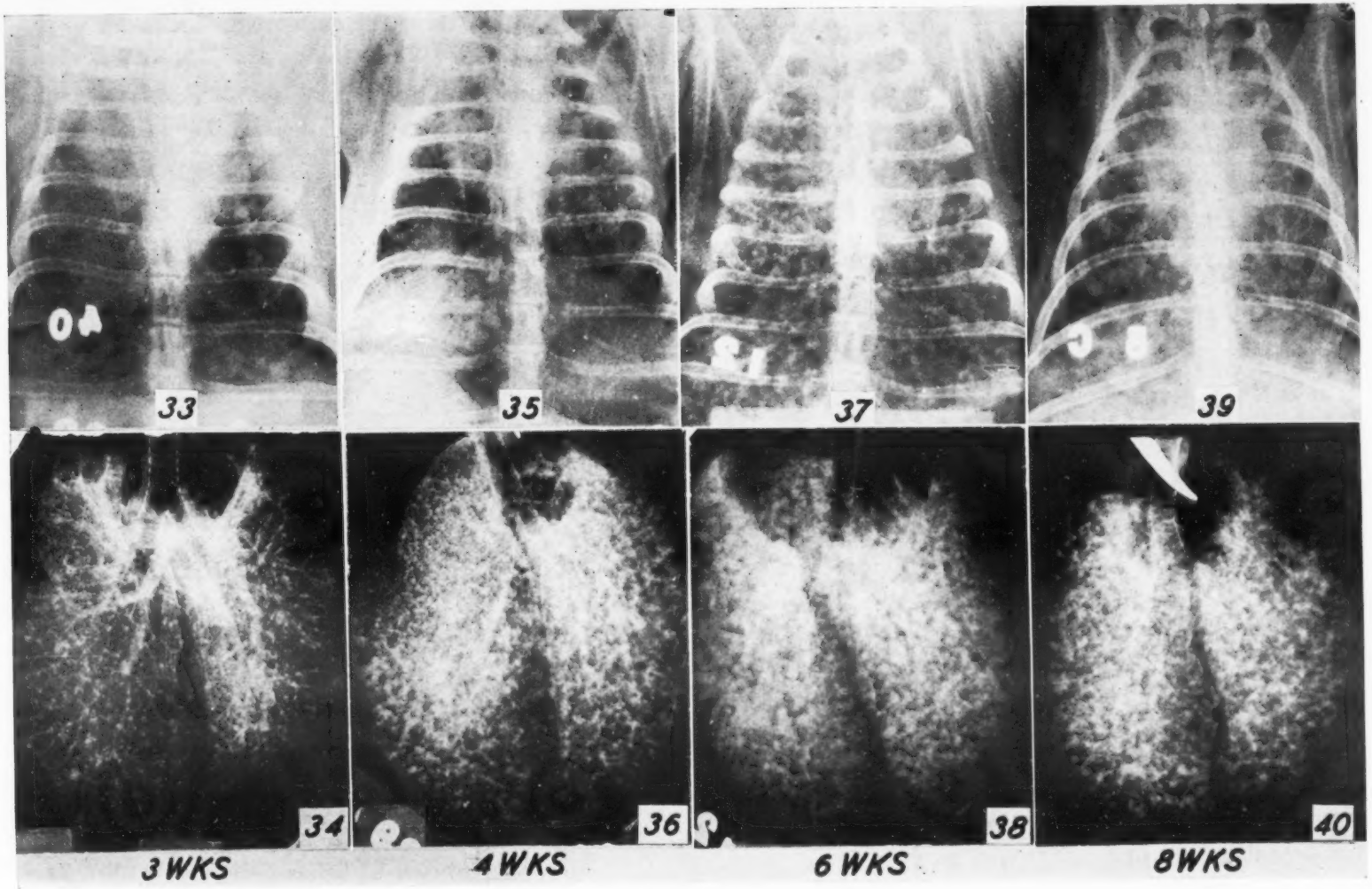


PLATE V

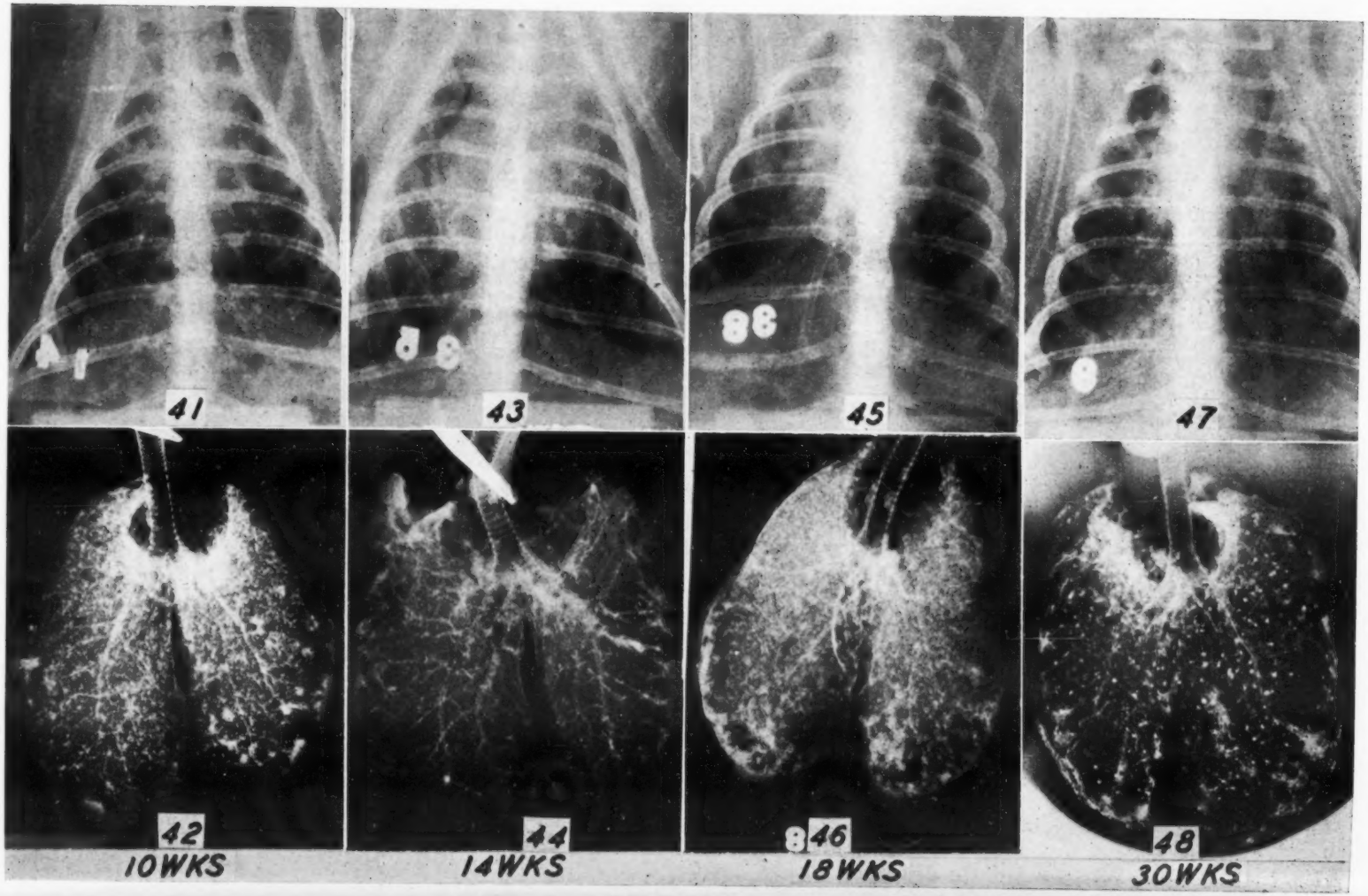


PLATE VI

PLATE VI

tion among the animals. Some of them showed no more disease at any time than is shown in figure 33, while others showed progression to a considerable degree, figure 37. Some of the animals showed "clearing" in the serial roentgenograms, that is, rabbits illustrated in figures 45 and 47 revealed as much disease at an earlier date as is shown in figure 39. In the serial roentgenograms of the rabbit illustrated in figure 47 the film taken just previous to the reinfection with virulent bacilli was similar to the picture shown in figure 29, indicating that at that time there was a considerable remnant of lesions from the vaccination. A comparison of the serial roentgenograms of the primary virulent infection with the reinfection series reveals the increased resistance engendered by the vaccination.

Roentgenograms of the excised lungs demonstrate, as in the previous group, considerably more disease than would be suspected from the chest roentgenograms. They also reveal the great irregularity in the pathological course and the presence of considerable disease in pulmonary areas adjacent to the diaphragm not evident in films of the thorax. At two weeks, figure 22, definite disease is revealed, a condition not shown in the other series at such a date. A careful comparison of the series with the primary virulent series shows that the pathological course differs in certain respects. The "snow storm" effect in the reinfection series is more on the order of sleet than of snow flakes during the fourth to the tenth week. Individual foci are more definite and when lesions are superimposed the resultant shadows are more dense in the reinfected series. The process of resolution and of progression which was discussed earlier in the paper is well shown in figures 42 to 48. Progressive disease is present in the dorsal and the higher caudal portions of the lung and the lesions in these locations are areas of tuberculous bronchopneumonia.

The lungs shown in figure 48 are of particular interest as they show the remnants of vaccination (dense circular shadows); resolving reinfection foci (faint shadows in the thicker portion of the lungs); and progressive reinfection lesions (the large irregular shadows) in some of which small, dense dots and lines indicate compact calcium deposit in caseous areas. It is quite likely that this pair of lungs resembled at an earlier date the ones shown in figure 46, if the dense vaccination lesions are disregarded, for the serial roentgenograms of the thorax of these two animals were similar up to the time one of them was sacrificed.

As in the other series, macroscopical inspection and microscopical study revealed considerably more disease than was shown in the roentgenograms. At two weeks, the involvement was as extensive as at three weeks in the primary virulent infection, and microscopical study showed that, while there was abundant inflammatory exudate and congestion, the cells were not closely packed. This probably accounts for the failure of the roentgenograms to show the real size of the foci and the volume of disease at this date. In the portions of the lungs where resolution eventually occurred few lesions showed abscess formation or caseation, the majority of lesions being composed of monocytes and lymphocytes with little or no new fibrous tissue formation. The lesions in the areas where progression occurred were indistinguishable, in the earlier phases, from

primary virulent lesions but at a later date fibrosis in the periphery and deposits of calcium in the old caseous foci were commonly found.

The histopathological picture found in the lungs shown in figure 48 is of particular interest for comparison with the roentgenograms of the excised lungs. The findings in the foci remaining from the vaccination were similar to those found in figure 30. A few of the lesions of reinfection resembled the lesions of vaccination except that fibrosis was not as extensive and no calcification was present. Most of the resolving lesions were composed of a rather loose aggregation of monocytes and lymphocytes. The large reinfection lesions showed a combination of areas of dense pus, of caseation, of calcium deposit in the caseous material, of fibrosis in the peripheral areas, of tuberculous abscesses opening into bronchi and of local bronchogenic "spreads." Eventually some of these lesions would have cavitated. Of particular interest is the fact that on the basis of density of shadow it was not possible to distinguish between fibrosis, caseation, dense pus and calcification (unless compact). The "soft" shadows represent loose-knit resolving foci. The very small, dense dots seen in the more voluminous part of the lungs represent small reinfection lesions with a dense "core" of caseation and with some capsule formation in the periphery.

DISCUSSION

When experimental animals are used to study tuberculous infection it must be borne in mind that the disease in man and in the animal cannot be regarded as identical. The type of animal used may be too susceptible or too resistant to the induced infection to allow the production of a chronic progressive disease similar to that found in adult human beings. In essense the pathogenesis of the disease is the same in man and animal but the soil in which the disease develops is different.

From our extensive studies of pulmonary tuberculosis in the rabbit, as produced by intravenous inoculation of bovine tubercle bacilli, we have determined that the course of the infection depends in very large part upon the virulence of the bacillus and upon the nature of the soil in which the infectious agent is planted, that is, normal or vaccinated soil. And in vaccinated animals the pulmonary disease bears a fairly close resemblance to that of man provided the dosage of virulent bacilli in the reinfection is small and the influence of posture (11, 12) is considered.

With regard to roentgenological studies the rabbit offers certain distinct advantages over man. Miliary pulmonary tuberculosis can be consistently produced at will and it should be possible to determine how soon after infection shadows (which will always be bilateral) can be demonstrated. A careful study of the actual pathological process at progressive intervals should reveal what factors are responsible for the casting of shadows. Progression of the disease produced by nonvirulent and by virulent bacilli can be studied to determine whether any essential difference exists in the type of shadow. The behavior of infection with virulent bacilli in virgin and vaccinated soil can also be studied to find out whether it is possible to differentiate between a primary infection and a reinfection by roentgenographic shadows alone.

Certain interesting facts have been determined in our study. Definite, though faint, shadows in roentgenograms of the thorax were first observed three weeks after infection. Shadows cast by lesions of nonvirulent and of virulent infection are indistinguishable and differentiation can be made only from serial roentgenograms of the thorax. The same is true with regard to the shadows of primary infection and reinfection with virulent bacilli. Shadows cast by residual lesions present six months to a year after infection with nonvirulent bacilli closely resemble the type of shadow seen in roentgenograms of the thorax at three weeks after virulent bacilli have been inoculated. Roentgenograms of excised lungs consistently revealed much more disease than was evident in comparative roentgenograms of the thorax and they also showed a difference in the type of disease in some instances. Neither the roentgenograms of the thorax nor those of the excised lungs revealed the involvement in volume or as early as did macroscopical inspection of the lung, and microscopical study revealed far more and earlier lesions than did any other method. If lesions were very numerous and small, a "snow storm" effect, from superimposition of lesions, was obtained that closely resembled the picture obtained when lesions were much fewer and larger. Caseation, *per se*, was not necessary for the production of a shadow. Congestion, sufficient in amount to be seen macroscopically, in and about tuberculous foci did not cast a shadow even in excised lungs where many small lesions were present. No shadows from tuberculous lesions were observed in any roentgenograms of the excised lungs if the involvement was less than ten days old.

A comparison of the histopathological appearance with the roentgenograms of the excised lungs and of the thorax gave the following impression relative to the factors responsible for the production of a shadow. An area of at least the volume of a primary pulmonary lobule must be packed densely with the cells of inflammation before an early lesion will become apparent, when "soft" ray technique is used to obtain a roentgenogram of excised and inflated lungs. Since the roentgenograms of the thorax, where a "hard" ray technique was employed, revealed much less disease it seems certain that the dense portion of individual foci must be considerably larger before a shadow will be cast. This means that by the time a parenchymal shadow of small size is seen an area of tuberculous bronchopneumonia is present. A larger lesion with only a loose arrangement of cells in the alveolar spaces would probably not cast a shadow of itself, but if superimposed upon lesions of a similar nature shadows, not representative of individual lesions, may be cast. Shadows may be cast by lesions which show no evidence of caseation. Old organized lesions of 1 mm. or more in diameter will cast a shadow if dense fibrotic tissue predominates. A lesion with a large caseous core and a relatively narrow fibrotic capsule will cast a shadow similar to a fibrotic tubercle with little or no caseous material present. If a lesion, as large as either type mentioned, is composed of irregular, loose-meshed fibrous tissue infiltrated loosely with lymphocytes and monocytes and with air-filled alveoli within its border, it is doubtful if any shadow will be cast. Old lesions, if of sufficient size and compactness, may cast shadows which might be interpreted as calcified lesions. This point is clearly shown by the "hard" shadows illustrated in the excised lungs with the large majority of these lesions failing to show any appre-

ciable calcium on microscopical examination. When calcium is deposited in amounts that can be demonstrated macroscopically, dense shadows are cast, as shown in the roentgenograms of excised lungs that contained old lesions of reinfection in which considerable calcification was present. Whether such small deposits of calcium could be revealed in roentgenograms of the thorax could not be determined in our study since the calcium-containing foci were situated in thoracic areas adjacent to the diaphragm.

From this comparative study the following deductions may be drawn: While size of lesion may play a part in determining whether a shadow will be cast, the compactness of the lesion is of far greater importance. "Hard" shadows may be cast by lesions containing dense fibrous tissue, dense caseous material without calcification or calcified caseous material. "Hard" shadows, *per se*, do not indicate that a lesion is "healed." A negative roentgenogram of the thorax or of excised lungs does not rule out the existence of small progressive tuberculous foci.

In general our results agree with the observation of other authors who have made investigations on experimental pulmonary tuberculosis, although they have incited a pneumonic focus of considerable size by introducing large numbers of bacilli through the air passages. Our studies have been directed toward the study of pneumonic foci which have been microscopical in size at the onset. We believe that the method we have used affords the better way to conduct an investigation of the limitations of roentgenography of the thorax.

Steiner, in his study of miliary pulmonary tuberculosis in man, did not resort to roentgenograms of the excised lungs because a different technique was necessary to obtain satisfactory roentgenograms and hence films of the thorax and of the excised lungs would not be comparable. We regret that Steiner did not make a roentgenological study of excised lungs, for it is probable that he would have shown much more involvement than was suspected from films of the thorax. It is important to apprise roentgenologists and the manufacturers of roentgenological apparatus and of X-ray films of the fact that roentgenography of the thorax with present technique may fail to reveal certain types of pulmonary pathological involvement. There is no better way to visualize the limitations of the present situation than to show the great contrast between tuberculous lungs *in situ* and excised.

It is, of course, thoroughly recognized that the problems of motion in the living and of certain features of the thorax, combined with the present physical limitations in X-ray apparatus, restrict the range of suitable roentgenological technique. These very difficulties should stimulate research to make available to the medical profession the materials and the technique that will enhance the value of roentgenography. There is no better approach to the improvement of roentgenological tools than to utilize an experimental method comparable to the experiments here reported. It is not our intent to deprecate the great value of roentgenography in the study of tuberculosis, but rather to draw attention to its present limitations in the hope that further improvements may ensue.

Steiner, in his study, was handicapped by the fact that he was unable to establish the age or the pathogenesis of the miliary lesions. We believe that the data

here presented will help to clarify the concept of miliary pulmonary tuberculosis in both a pathological and radiological sense.

We (11) have demonstrated tubercle bacilli in a small percentage of individuals who have had normal roentgenograms of the chest. It is quite probable, though not possible of proof, that these persons had small parenchymal lesions which were undetectable by accepted roentgenological technique. In a tuberculosis sanatorium it is not uncommon to discover small "spreads" of the disease to new areas of the lung parenchyma. When these are first demonstrated it is probable that the actual "spread" occurred two weeks or more prior to its detection in the chest film. This, of course, does not apply so much to massive "spreads" where the shadows observed may be caused in part by the necrotic, purulent or haemorrhagic material that has been inhaled. "Clearing" of parenchymal areas is a common observation when serial roentgenograms of the chest of patients are available. It should be constantly borne in mind that it is possible to have undemonstrable tuberculous foci still present in the cleared area when the usual roentgenograms are relied upon. Planigraphic films sometimes reveal tuberculous foci of considerable volume not suspected when stereo-roentgenograms are studied. Frequently patients with chronic tuberculosis will be regarded as having unilateral disease because one lung field appears clear and upon such an interpretation a lobectomy or a pneumonectomy will be considered with the idea that this operation will eradicate the pulmonary infection. When such a problem is under discussion due consideration must be allowed for the possibility of existing tuberculous foci in the lung thought to be free of disease.

It is a very common practice for interpreters of roentgenograms of the chest to use such pathological terms as "fibrotic," "fibrocaseous," "calcified," "productive," "exudative," etc. From our experimental study it seems doubtful, except in instances where considerable calcium deposits are present, that such pathological interpretations are correct when shadows seen in single roentgenograms of the chest are interpreted. When serial roentgenograms are available such interpretations may be allowable, provided that it is remembered that readings are based upon inference and not upon shadow characteristics that are pathognomonic of the lesions interpreted. We suggest that it would be a better practice if interpretations of intrathoracic shadows observed in roentgenograms of the chest are limited to descriptions of the location, extent of distribution and density of shadows and to forego any pathological interpretation until a satisfactory roentgenological classification has been evolved which will clearly differentiate the processes mentioned above.

SUMMARY

A comparative study of the pathological findings and of roentgenograms of the thorax and of inflated excised lungs in miliary pulmonary tuberculosis in the rabbit is presented. Primary infection with nonvirulent and with virulent bovine tubercle bacilli and reinfection with virulent bacilli in animals vaccinated with nonvirulent organisms were studied.

The study revealed that the greatest amount and the essential nature of patho-

logical lesions were best determined, as would be expected, by microscopical examination. To a lesser degree the pathological findings were demonstrable in the following sequence: macroscopical examination, roentgenograms of excised lungs and, last, roentgenograms of the thorax.

By shadows alone single roentgenograms of the thorax failed to distinguish between primary infections with virulent and with nonvirulent bacilli or between primary and reinfection with virulent bacilli. Serial roentgenograms gave a clue as to the type of infection but not through the nature of the shadows *per se*.

Dense noncaseous, caseous, caseo-fibrotic and fibrotic areas could not be differentiated in roentgenograms of inflated excised lungs. Compact deposits of calcium, recognizable by touch or by contact with a knife, were clearly shown in caseous foci but with lesser deposits no differentiation between caseous material and calcium was evidenced.

Superimposition of lesions causes a density in shadows that does not indicate their true nature. Compactness rather than size is the most important factor in determining whether a shadow of a focus will be cast. It appears that the compact portion of a lesion must be equal to at least the volume of a primary parenchymal lobule before a shadow will be cast in roentgenograms of excised lungs and that shadows, when first observed in roentgenograms of the thorax, indicate foci with considerably larger areas of compact, inflammatory exudate. This suggests that the loosely-arranged periphery, the so-called "collateral" inflammation may not cast a shadow and that the true size of a tuberculous focus is not revealed by a roentgenographic shadow. If these facts are applicable to shadows observed in roentgenograms of the human chest, then it would seem that the earliest demonstrable tuberculous focus would be a compact area of tuberculous bronchopneumonia, which has all the spreading potentialities characteristic of any type of bronchopneumonia.

SUMARIO

Este estudio comparado versa sobre los hallazgos patológicos y los roentgenogramas del tórax y de los pulmones extirpados inflados en la granulia del conejo, habiéndose estudiado la infección primaria por bacilos tuberculosos bovinos avirulentos y virulentos y la reinfección con bacilos virulentos en animales vacunados con microbios avirulentos.

El estudio reveló que, según era de esperar, la mejor manera de determinar el mayor número y naturaleza èsencial de las lesiones patológicas, era por medio del microscopio. En menor grado pudieron descubrirse los hallazgos patológicos en el siguiente orden: examen macroscópico, roentgenogramas de los pulmones resentidos y finalmente en los roentgenogramas del tórax.

Por medio de las sombras exclusivamente los roentgenogramas aislados del tórax no pudieron diferenciar las infecciones primarias por bacilos virulentos y avirulentos y la infección primaria y la reinfección por bacilos virulentos. Los roentgenogramas seriados facilitaron alguna idea del tipo de la infección, pero no fundada en la naturaleza de las sombras en sí mismas.

En los roentgenogramas de los pulmones extirpados inflados no pudieron diferenciarse las zonas espesas no caseosas, caseosas, caseo-fibrosas y fibrosas.

En los focos caseosos se observaron claramente depósitos compactos de calcio reconocibles al tacto o por el contacto con un cuchillo, pero cuando se trataba de depósitos mas pequeños no podían diferenciarse las sustancias caseosas, del calcio.

La sobreposición de las lesiones provoca en las sombras un espesor que no indica su verdadera naturaleza. La densidad más bien que el tamaño constituye el factor mas importante para determinar si un foco lanzará o no sombra. Según parece, la porción compacta de una lesión debe ser por lo menos igual al volumen del lóbulo primario del parénquima antes de que lance una sombra en las radiografías de los pulmones extirpados y que cuando se observan por primera vez sombras en las radiografías torácicas indican la presencia de focos con zonas mucho mayores de exudado inflamatorio compacto, lo cual indica que la periferia floja, la llamada inflamación "colateral" tal vez no lance sombra y que una sombra radiográfica quizás no revele el verdadero tamaño de un foco tuberculoso. Si esos datos tienen aplicación a las sombras observadas en los roentgenogramas del tórax humano parece que el primer foco tuberculoso demostrable debe consistir en una sombra compacta de bronconeumonía tuberculosa que posee todas las potencialidades de propagación que caracterizan a cualquier tipo de bronconeumonía.

The authors are deeply indebted to E. C. Lasher, R. T., for the roentgenograms of the thorax and of the excised lungs and for the preparation of the illustrations which accompany the article.

REFERENCES

(1) Dunham, H. K., and Skavlem, J. H.: A comparative study of the pathology and X-ray densities of tuberculous lung lesions, Am. Rev. Tuberc., 1921–22, *5*, 278.

(2) Opie, E. L., and Andersen, H.: First infection with tuberculosis by way of the lungs, Am. Rev. Tuberc., 1920, *4*, 629.

(3) Opie, E. L.: The use of X-rays in the postmortem examination of lungs with reference to tuberculosis, J. Tech. Methods, 1941, *21*, 1.

(4) Miller, G. R.: The recognition of thoracic calcifications, Am. J. Roentgenol., 1931, *26*, pt. 1, 191.

(5) Steiner, P. E.: The histopathological basis for the X-ray diagnosability of miliary pulmonary tuberculosis, Am. Rev. Tuberc. 1937, *36*, 692.

(6) Soper, W. B., Sampson, H. B., and Haskins, C. H.: Aspiration tuberculosis in rabbits, Am. Rev. Tuberc., 1927, *15*, 88.

(7) Burke, H. E.: A comparison of the roentgenological and pathological findings in experimental pulmonary tuberculosis in rabbits, Am. Rev. Tuberc., 1935, *32*, 343.

(8) Mills, M. A., Barth, E. E., and Gunn, F. D.: Experimental pulmonary tuberculosis in the dog, Am. Rev. Tuberc., 1940, *42*, 28.

(9) Medlar, E. M., and Sasano, K. T.: An interpretation of the pathology of pulmonary tuberculosis, Am. Rev. Tuberc., 1931, *24*, 705.

(10) Medlar, E. M., and Sasano, K. T.: The effects of virulence of microorganisms on the histopathology of experimental pulmonary tuberculosis, Am. Rev. Tuberc., 1933, *28*, 62.

(11) Medlar, E. M., and Sasano, K. T.: A study of the pathology of experimental pulmonary tuberculosis in the rabbit, Am. Rev. Tuberc., 1936, *34*, 456.

(12) Medlar, E. M.: Pulmonary tuberculosis in cattle: The location and type of lesions in naturally acquired tuberculosis, Am. Rev. Tuberc., 1940, *41*, 283.

(13) Medlar, E. M., Ordway, W. H., and Pesquera, G. S.: Acid-fast bacilli in patients of a nontuberculous medical service, Am. Rev. Tuberc., 1943, *48*, 304.

PATIENTS DISCHARGED ALIVE FROM A COUNTY SANATORIUM[1]

D. R. HASTINGS AND BORGHILD BEHN

At no time in the history of the control of tuberculosis has there been a greater need than there is to-day for an evaluation of the methods for the treatment and care of the tuberculous patient. During the past few years there has been considerable interest aroused in the postsanatorium morbidity and mortality experience of tuberculous patients. Of unusual interest to both the patient and the physician is the effect of collapse therapy on the postsanatorium progress of the discharged patient. There have been numerous studies in which some attempt has been made to evaluate pneumothorax and other forms of collapse therapy, but it is quite apparent that a completely sound statistical approach to the problem has not yet been and perhaps cannot be attained. Unfortunately it is not possible to select cases in advance and divide them into groups matched perfectly in every respect, except as to the type of treatment to be given. No matter how careful the *postfacto* classification of cases, one cannot be certain that there may not be some bias in selection which might tend to vitiate or obscure the results obtained in comparison of the effects of the various types of treatment. All we can hope is that, if we study a sufficiently large number of cases, the various unknown or uncontrollable factors which might possibly affect the patients' postsanatorium progress will be operative to approximately the same degree in each of the groups to be compared. The results obtained by various investigators are far from being in complete harmony, and in some cases suffer fairly obviously from bias due to preconceived notions on the part of the investigator. As a matter of fact, no guarantee can be given that the evidence to be presented in this paper will be conclusive, or even that it will prove perfectly free from bias. Our interest in the problem was intensified by the results obtained in a study of clinic cases which we did a year or so ago. Somewhat to our surprise we found no differences in mortality between pneumothorax cases attending clinic for refills and the noncollapse cases attending the other tuberculosis clinics under the supervision of the Out-Patient Department. We found a much larger percentage of readmissions to the Sanatorium among the cases who had collapse therapy prior to discharge. The present investigation is an attempt to determine whether the results obtained previously were peculiar to the selected sample of clinic patients under consideration or whether they would still hold for all patients with pulmonary tuberculosis discharged alive from the Sanatorium.

This study is based on the records of 3,797 patients with adult type pulmonary tuberculosis discharged alive from Glen Lake Sanatorium during the twenty-five year period from 1916, when the Sanatorium was opened, through 1940. The cases are divided into three groups for comparison: (*1*) those with no collapse therapy prior to their last live discharge, (*2*) those who have had pneumothorax only, and (*3*) those who have had some other form of collapse therapy, either alone or in

[1] From Glen Lake Sanatorium, Oak Terrace, Minnesota.

combination with pneumothorax. The unsuccessful pneumothorax cases, except those who subsequently had some other type of surgical treatment, are included with the group having no surgery.

We know that the stage of disease and condition of the patient on discharge are important in determining the progress of the patient after discharge from the Sanatorium. It is necessary, therefore, to classify each of the three groups to be compared according to stage of disease and condition on last live discharge, in order that we may be reasonably certain that whatever difference in their post-sanatorium history may show up can be ascribed to differences in treatment prior to discharge and not to these other factors. The classification of the three groups according to stage of their tuberculosis on discharge is shown in table 1. There is a much larger proportion of far advanced cases among those who had had collapse therapy than in the group without any type of chest surgery. Knowing that the prognosis for far advanced cases of tuberculosis is much less favorable than for those whose tuberculosis is minimal or moderately advanced at discharge

TABLE 1

	NO SURGERY		PNEUMOTHORAX		OTHER SURGERY		TOTAL	
	Number	Per Cent	Number	Per cent	Number	Per cent	Number	Per cent
Minimal	484	20.2	25	3.9	20	2.6	529	13.9
Moderately advanced	979	40.8	278	43.6	230	30.2	1,487	39.2
Far advanced	935	39.0	335	52.5	511	67.2	1,781	46.9
Total	2,398	100.0	638	100.0	761	100.0	3,797	100.0

one would expect, then, a higher mortality among the cases with collapse therapy than among those who have not had surgical treatment.

The distribution of cases within the three groups classified according to condition on last live discharge is given in table 2.

From the percentages quoted in table 2 it seems evident that on the whole the effect of collapse therapy prior to discharge has been favorable. The progress of the disease has been arrested in a somewhat larger proportion of those patients who had some form of surgical treatment, and a very much smaller percentage of these patients left the Sanatorium with their tuberculosis unimproved. We know that patients whose tuberculosis is arrested at the time of discharge from the Sanatorium have a much better postsanatorium prognosis than those in whom the tuberculous process is still active. Consequently, in our analysis of the post-sanatorium progress of these tuberculous patients we must recognize that the groups with collapse therapy have an initial advantage over the group which had no surgical treatment. Our concern here is to determine whether, when condition on discharge is held constant, the collapse therapy groups maintain this advantage over the group which received no treatment other than bed-rest while they were at the Sanatorium.

So far, we have considered two factors which might influence the progress of the patient after his discharge from the Sanatorium and have found that they affect the three groups to be compared differentially. It is important therefore that, in any comparison of morbidity and mortality statistics between the collapse and noncollapse groups, the stage of the disease and the condition of the patient on discharge be taken into account.

Table 3 shows the status in 1941 of the 3,797 tuberculous patients who have had at least one discharge alive during the twenty-five year period under consideration.

TABLE 2

	No surgery		Pneumothorax		Other surgery		Total	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Arrested	735	30.7	287	45.0	303	39.8	1,325	34.9
Apparently arrested	188	7.8	46	7.2	23	3.0	257	6.8
Quiescent	348	14.5	66	10.4	102	13.4	516	13.6
Improved	572	23.9	200	31.3	270	35.5	1,042	27.4
Unimproved	555	23.1	39	6.1	63	8.3	657	17.3
Total	2,398	100.0	638	100.0	761	100.0	3,797	100.0

TABLE 3

	No surgery		Pneumothorax		Other surgery		Total	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Untraced	731	30.5	146	22.9	156	20.5	1,033	27.2
Dead	876	36.5	93	14.6	153	20.1	1,122	29.5
Alive	791	33.0	399	62.5	452	59.4	1,642	43.3
Total	2,398	100.0	638	100.0	761	100.0	3,797	100.0

The information on the present condition of these patients is obtained from several sources: from replies to follow-up letters sent each year on the anniversary of the patient's discharge, from the patient's private physician whenever possible, from clinic contacts, and through home visits by the Out-Patient Department nurse and social worker. It is interesting to note that information has been obtained concerning a larger proportion of the collapse than of the noncollapse cases. Apparently, as a group, they are somewhat more coöperative in that respect. Those alive and traced in 1941 will be studied in more detail later, with special attention to the working capacity of these patients. Any direct comparison of the percentage of deaths in the three groups at this point would have little value. We have no way of determining what proportion of those whom we were unable to trace in 1941 are dead, and other factors such as stage of disease and

condition on discharge, and length of time since discharge should also be taken into consideration. Only those ex-patients who have been followed for at least a year subsequent to their discharge from the Sanatorium, and whose status in 1941 is known, will be included in the final analyses. There were in all 2,764 persons traced in 1941, 1,667 with no collapse therapy prior to discharge from the Sanatorium, 492 with pneumothorax alone and 605 with some other surgical treatment, either alone or in combination with pneumothorax.

One criterion which might be used in evaluating the effect of treatment is the number of years which elapse before the death of the patient after his discharge from the Sanatorium. This is admittedly a crude measure because there are too many uncontrollable factors which might possibly affect the results. All we can hope is that whatever factors are operating will affect the three groups to be compared in approximately the same way. The groups are roughly comparable in their age distribution at the time of discharge, though there may have been some

TABLE 4

Deaths from all causes

	NO SURGERY		PNEUMOTHORAX		OTHER SURGERY		TOTAL	
	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$
Minimal	40	7.25	1	1.00	4	1.75	45	6.62
Moderately advanced	289	3.47	23	2.74	43	3.14	355	3.39
Far advanced	547	2.23	69	2.43	106	2.25	722	2.25
Arrested	124	4.70	14	2.64	26	2.88	164	4.24
Not arrested	752	2.57	79	2.47	127	2.41	958	2.54
Total	876	2.87	93	2.49	153	2.49	1,122	2.79

slight tendency to refrain from using collapse therapy in the older age groups. As far as the patients' social and economic condition after leaving the Sanatorium are concerned, previous investigations have shown that, for clinic patients at least, the differences between collapse and noncollapse cases are not great enough to be of any practical significance. We do know there are differences between these groups in stage of disease and condition of the patient at the time of his last live discharge from the Sanatorium, but these are factors which can be taken into account.

Table 4 gives the average number of years which elapsed between date of last live discharge from the Sanatorium and date of death in each of the three groups. Deaths from all causes are classified according to stage of disease and condition of the patient at the time of his last discharge alive. Table 5 presents the same information for those cases in which tuberculosis was given as the cause of death.

In tables 4 and 5 the symbol "$\bar{x}$" is used to represent the average number of years elapsed between date of discharge and date of death. The Analysis of Variance technique has been used to determine whether there are significant

differences between the groups in average number of years of life after discharge. However, even a cursory inspection of the averages in the tables is enough to indicate that there are no real differences between the collapse and noncollapse groups. There appears to be a very large difference between the collapse and noncollapse cases who were discharged with minimal tuberculosis, but this must be discounted because the number of cases in this classification was very small. The difference of approximately 1.5 years duration of life after discharge between the collapse and noncollapse groups for those cases whose tuberculosis was arrested on discharge also proved to be statistically insignificant, whether deaths from all causes or deaths from tuberculosis alone were considered. In the "No Surgery" group, stage of disease and condition of the patient on discharge seem to be quite important in determining duration of life after discharge. All of the differences observed in this group are highly significant. Apparently those

TABLE 5

Deaths from tuberculosis

	NO SURGERY		PNEUMOTHORAX		OTHER SURGERY		TOTAL	
	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$	N	$\bar{x}$
Minimal	19	6.16	1	1.00	2	2.50	22	5.59
Moderately advanced	217	3.03	16	2.88	30	2.73	263	2.98
Far advanced	491	1.96	59	2.59	86	1.93	636	2.02
Arrested	68	4.28	10	2.80	11	2.73	89	3.92
Not arrested	659	2.20	66	2.61	107	2.08	832	2.21
Total	727	2.39	76	2.63	118	2.14	921	2.38

whose tuberculosis was classified as minimal at the time of their last live discharge lived about twice as long after discharge as those who had moderately advanced and three times as long as those who had far advanced tuberculosis on discharge from the Sanatorium. Those who were classified as arrested or apparently arrested lived about two years longer on the average than those whose tuberculosis was quiescent, improved or unimproved on discharge. The conclusions are the same whether deaths from all causes or deaths from tuberculosis alone are considered. These results agree with those found in other investigations. However, the results of similar comparisons in the groups who had collapse therapy prior to discharge proved somewhat surprising. Stage of disease and condition of the patient on discharge seem to have had little or no effect on duration of life after discharge. The average number of years elapsed between date of discharge and date of death seem to be approximately the same for all classifications. Of course it must be remembered that the actual number of deaths in the collapse groups was very small. It is quite possible that if a longer

series were available the results might be more nearly in accord with those found among the noncollapse cases.

While pneumothorax was first used at the Sanatorium in 1916 and the first thoracoplasty was performed in 1922, some form of collapse therapy was used in only 12.4 per cent of the patients discharged during the period from 1916 through 1930, in contrast with 53.4 per cent of the group discharged after 1930. Comparison of the crude death rates for the twenty-five year period as a whole might prove somewhat misleading since there has been a change in the proportion of cases given collapse therapy from 1916 to 1940. Unfortunately the total number of cases available is hardly large enough to justify the calculation of age specific death rates, but we may safely assume that the differences in age distribution of the collapse and noncollapse groups are not large enough to be of any practical significance. Comparison between the collapse and noncollapse groups for death rates will be made for two periods, 1916–1930 and 1931–1940, as well as for the twenty-five year period as a whole. A fifteen- and a ten-year period as bases for calculating death rates may seem rather long, but if the rates were calculated for a shorter period the number of cases in each subgroup would be reduced to the point where any difference observed could be ascribed to the fluctuations of sampling. The death rates have been made specific for stage of disease and condition of the patient at the time of his last discharge alive from the Sanatorium. As has been pointed out before, these two factors are important in prognosticating the postsanatorium history of the discharged patient, and we have shown that the distribution of cases with respect to stage of disease and condition on discharge are not the same for the collapse and noncollapse groups.

Only the persons traced in 1941 are included in the following analyses of death rates. We have no way of determining whether all of the persons in the untraced groups are still alive, or whether a certain proportion of them may be dead. It seems most reasonable to assume that the proportion of deaths would be about the same as for those who were traced in 1941 in each of the three groups to be compared. In other words, we are assuming that whatever conclusions may be reached from a consideration of only the persons traced in 1941 would not be changed if we had information as to whether those not traced were alive or dead in 1941. Table 6A gives the percentage of deaths from all causes in each of the three groups by date of last live discharge and stage of disease on discharge. The percentages of deaths from tuberculosis alone are presented in table 6B. In applying tests for significance of differences between the percentage of deaths for the collapse and noncollapse groups, the minimal and moderately advanced cases have been combined because collapse therapy was used so seldom for the treatment of minimal cases of tuberculosis. The results of the tests are presented in table 6C.

The information presented in tables 7A, 7B, 7C is similar to that given in tables 6A, 6B, 6C, except that the patients in the various groups have been classified according to their condition on discharge instead of according to the stage of their tuberculosis. For purposes of comparison between the collapse and non-

TABLE 6A

Per cent of deaths from all causes

	NO SURGERY			PNEUMOTHORAX			OTHER SURGERY			TOTAL		
	N_L	N_D	P	N_L	N_D	P	N_L	N_D	P	N_L	N_L	P
1916–1930												
Minimal	102	32	31.4	1	1		1	0		104	33	31.7
Moderately advanced	331	210	63.4	30	12	40.0	20	12	60.0	381	234	61.4
Far advanced	458	404	88.2	54	27	50.0	27	14	51.9	539	445	82.6
Total	891	646	72.5	85	40	47.1	48	26	54.2	1,024	712	69.5
1931–1940												
Minimal	147	8	5.4	19	0	0.0	14	4		180	12	6.7
Moderately advanced	330	79	23.9	170	11	6.5	160	31	19.4	660	121	18.3
Far advanced	299	143	47.8	218	42	19.3	383	92	24.0	900	277	30.8
Total	776	230	29.6	407	53	13.0	557	127	22.8	1,740	410	23.6
1916–1940												
Minimal	249	40	16.1	20	1	5.0	15	4	26.7	284	45	15.8
Moderately advanced	661	289	43.7	200	23	11.5	180	43	23.9	1,041	355	34.1
Far advanced	757	547	72.3	272	69	25.4	410	106	25.9	1,439	722	50.2
Total	1,667	876	52.0	492	93	18.9	605	153	25.3	2,764	1,122	40.6

TABLE 6B

Per cent of deaths from tuberculosis alone

	NO SURGERY			PNEUMOTHORAX			OTHER SURGERY			TOTAL		
	N_L	N_D	P	N_L	N_D	P	N_L	N_D	P	N_L	N_D	P
1916–1930												
Minimal	102	15	14.7	1	1		1	0		104	16	15.4
Moderately advanced	331	169	51.1	30	9	30.0	20	6	30.0	381	184	48.3
Far advanced	458	373	81.4	54	26	48.1	27	12	44.4	539	411	76.2
Total	891	557	62.5	85	36	42.3	48	18	37.5	1,024	611	59.7
1931–1940												
Minimal	147	4	2.7	19	0	0.0	14	2		180	6	3.3
Moderately advanced	330	48	14.5	170	7	4.1	160	24	15.0	660	79	12.0
Far advanced	299	118	39.5	218	33	15.1	383	74	19.3	900	225	25.0
Total	776	170	21.9	407	40	9.8	557	100	18.0	1,740	310	17.7
1916–1940												
Minimal	249	19	7.6	20	1	5.0	15	2	13.3	284	22	7.7
Moderately advanced	661	217	32.8	200	16	8.0	180	30	16.7	1,041	263	25.3
Far advanced	757	491	64.9	272	59	21.7	410	86	21.0	1,439	636	44.2
Total	1,667	727	43.6	492	76	15.4	605	118	19.5	2,764	921	33.3

TABLE 6C

	DEATHS FROM ALL CAUSES		DEATHS FROM TUBERCULOSIS	
	Diff. ± S.E.$_{Diff.}$	k	Diff. ± S.E.$_{Diff.}$	k
NS—Pn.				
1916–1930				
Minimal + moderately advanced	14.0 ± 9.2	1.5	10.3 ± 9.2	1.1
Far advanced	38.2 ± 5.5	6.9*	33.3 ± 6.1	5.5*
Total	25.4 ± 5.2	4.9*	20.2 ± 5.6	3.6*
1931–1940				
Minimal + moderately advanced	12.4 ± 3.1	4.0*	7.2 ± 2.6	2.8*
Far advanced	28.5 ± 4.1	7.0*	24.4 ± 3.9	6.3*
Total	16.6 ± 2.6	6.4*	12.1 ± 2.3	5.3*
1916–1940				
Minimal + moderately advanced	25.3 ± 3.5	7.2*	18.2 ± 3.1	5.9*
Far advanced	46.9 ± 3.5	13.4*	43.2 ± 3.5	12.3*
Total	33.1 ± 2.5	13.2*	28.2 ± 2.4	11.7*
NS—S				
1916–1930				
Minimal + moderately advanced	−1.2 ± 11.1	0.1	13.9 ± 11.0	1.3
Far advanced	36.3 ± 7.5	4.8*	37.0 ± 8.4	4.4*
Total	18.3 ± 6.8	2.7*	25.0 ± 7.3	3.4*
1931–1940				
Minimal + moderately advanced	−1.9 ± 3.3	0.6	−4.0 ± 2.7	1.5
Far advanced	23.8 ± 3.6	6.6*	20.2 ± 3.3	6.7*
Total	6.8 ± 2.4	2.8*	3.9 ± 2.1	1.9
1916–1940				
Minimal + moderately advanced	12.1 ± 3.6	3.4*	9.5 ± 3.2	3.0*
Far advanced	46.4 ± 3.1	15.0*	43.9 ± 3.5	14.2*
Total	26.7 ± 2.3	11.6*	24.1 ± 2.2	11.0*

collapse groups, condition on discharge has been divided into categories. "Arrested" includes also those who were apparently arrested, while "Not Arrested" includes those patients whose condition on discharge was classified as quiescent, improved or unimproved.

The following is an explanation of the symbols used in tables 6 and 7:

NS	No Surgery—the group which had no collapse therapy prior to discharge
Pn.	Pneumothorax—the group which had pneumothorax established with at least partial success prior to discharge from the Sanatorium
S	Other Surgery—the group which had some other type of collapse therapy, either alone or in combination with pneumothorax

N_L Number discharged alive from the Sanatorium during the period in question

N_D Number dead by 1941 of those discharged alive

P Percentage of deaths $P = 100\frac{N_D}{N_L}$ $Q = 100 - P$

Diff. The indicated difference in percentage deaths

S.E.$_{Diff.}$ The standard error of the difference in percentages, calculated by the usual formula

$$S.E._{Diff.} = \frac{PQ}{N_{L_1}} + \frac{PQ}{N_{L_2}}$$

where $P = \frac{N_{D_1} + N_{D_2}}{N_{L_1} + N_{L_2}}$

k The ratio of the difference in percentages to its standard error. If k is greater than 2.0, the probability that the difference is due solely to the errors of sampling is less than 0.05, and the difference is said to be statistically significant. In tables 6C and 7C the significant differences are all starred.

TABLE 7A

Per cent of deaths from all causes

	NO SURGERY			PNEUMOTHORAX			OTHER SURGERY			TOTAL		
	N_L	N_D	P	N_L	N_D	P	N_L	N_D	P	N_L	N_D	P
1916–1930												
Arrested	144	43	29.9	7	2		4	1		155	46	29.7
Apparently arrested	72	27	37.5	3	0		1	0		76	27	35.5
Quiescent	152	99	65.1	6	3		6	6		164	108	65.9
Improved	180	153	85.0	58	25	43.1	32	15	46.9	270	193	71.5
Umimproved	343	324	94.5	11	10		5	4		359	338	94.2
1931–1940												
Arrested	339	47	13.9	223	9	4.0	221	24	10.9	783	80	10.2
Apparently arrested	38	7	18.4	34	3	8.8	20	1	5.0	92	11	12.0
Quiescent	82	26	31.7	48	6	12.5	82	21	25.6	212	53	25.0
Improved	207	72	34.8	80	24	30.0	186	48	25.8	473	144	30.4
Unimproved	110	78	70.9	22	11	50.0	48	33	68.8	180	122	67.8
1916–1940												
Arrested	483	90	18.6	230	11	4.8	225	25	11.1	938	126	13.4
Apparently arrested	110	34	30.9	37	3	8.1	21	1	4.8	168	38	22.6
Quiescent	234	125	53.4	54	9	16.7	88	27	30.7	376	161	42.8
Improved	387	225	58.1	138	49	35.5	218	63	28.9	743	337	45.4
Umimproved	453	402	88.7	33	21	63.6	53	37	69.8	539	460	85.3

On the basis of the tests of significance summarized in tables 6C and 7C, it appears fairly clear that chances of survival after discharge are better for the patients who had collapse therapy prior to discharge than for those who had bed-rest alone. The differences are not quite so marked for the group of minimal and moderately advanced cases but even here they are statistically significant for the

twenty-five year period as a whole. It also appears that for those whose tuberculosis was arrested or apparently arrested at the time of their last live discharge from the Sanatorium the chances of survival were about the same no matter what type of treatment they had received while at the Sanatorium. Here too, the differences between the percentages were statistically significant for the twenty-five year period as a whole, but that is probably due in part at least to the fact that relatively fewer of the cases were treated by collapse in the earlier years covered by this study. The average number of years exposed to the risk of death

TABLE 7B

Per cent of deaths from tuberculosis alone

	NO SURGERY			PNEUMOTHORAX			OTHER SURGERY			TOTAL		
	N_L	N_D	P	N_L	N_D	P	N_L	N_D	P	N_L	N_D	P
1916–1930												
Arrested	144	22	15.3	7	2		4	0		155	24	15.5
Apparently arrested	73	18	25.0	3	0		1	0		76	18	23.7
Quiescent	152	81	53.3	6	3		6	4		164	88	53.7
Improved	180	136	75.6	58	21	36.2	32	10	31.3	270	167	61.9
Unimproved	343	300	87.5	11	10		5	4		359	314	87.5
1931–1940												
Arrested	339	25	7.4	223	6	2.7	221	10	4.5	783	41	5.2
Apparently arrested	38	3	7.9	34	2	5.9	20	1	5.0	92	6	6.5
Quiescent	82	14	17.1	48	5	10.4	82	18	22.0	212	37	17.4
Improved	207	58	28.0	80	18	22.5	186	39	21.0	473	115	24.3
Unimproved	110	70	63.6	22	9	40.9	48	32	66.7	180	111	61.7
1916–1940												
Arrested	483	47	9.7	230	8	3.5	225	10	4.4	938	65	6.9
Apparently arrested	110	21	19.1	37	2	5.4	21	1	4.8	168	24	14.3
Quiescent	234	95	40.6	54	8	14.8	88	22	25.0	376	125	33.2
Improved	387	194	50.1	138	39	28.3	218	49	22.5	743	282	38.0
Unimproved	453	370	81.7	33	19	57.6	53	36	67.9	539	425	78.8

is probably somewhat less for the collapse groups than for the noncollapse group. However, as far as the patients with far advanced tuberculosis or those in whom the tuberculous process was still active at the time of their last live discharge from the Sanatorium are concerned, there can be little question that their chances of survival were much better if they had had collapse therapy prior to discharge. The percentage of deaths was considerably greater in the noncollapse group than in either of the two collapse groups for those discharged alive between 1916 and 1930 as well as for those discharged alive between 1931 and 1940 and for the twenty-five year period as a whole. In general the differences are slightly smaller in magnitude, although in no instance by enough to affect the conclusions, when

deaths from tuberculosis alone are considered. It is, however, still possible that the differences are not quite so marked as they appear to be here. It should be remembered that on the average the collapse groups have not been exposed to the risk of death as long as those who have had no surgery. It has been pointed out previously that the proportion of cases receiving collapse therapy has steadily increased from 1916 to 1940, and these differences in length of time since discharge

TABLE 7C

	DEATHS FROM ALL CAUSES		DEATHS FROM TUBERCULOSIS	
	Diff. ± S.E.$_{Diff.}$	k	Diff. ± S.E.$_{Diff.}$	k
NS—Pn.				
1916–1930				
Arrested	12.4 ± 15.3	0.8	−1.5 ± 12.8	0.1
Not arrested	34.6 ± 4.8	7.2*	31.3 ± 5.5	5.7*
1931–1940				
Arrested	9.6 ± 2.5	3.8*	4.3 ± 1.8	2.4*
Not arrested	16.8 ± 4.6	3.7*	14.3 ± 4.4	3.3*
1916–1940				
Arrested	15.7 ± 2.6	6.0*	7.8 ± 2.0	3.9*
Not arrested	34.9 ± 3.6	9.7*	32.1 ± 3.7	8.7*
NS—S				
1916–1930				
Arrested	12.4 ± 21.4	0.6	18.5 ± 17.9	1.0
Not arrested	27.2 ± 6.2	4.4*	34.7 ± 7.1	4.9*
1931–1940				
Arrested	−1.9 ± 3.3	0.6	14.0 ± 2.7	1.5
Not arrested	23.8 ± 3.6	6.6*	20.2 ± 3.3	6.7*
1916–1940				
Arrested	10.3 ± 2.7	3.8*	7.0 ± 2.1	3.3*
Not arrested	34.6 ± 3.0	11.5*	31.6 ± 3.0	10.5*
S—Pn.				
1916–1930	7.1 ± 8.3	0.9	−4.8 ± 8.9	0.5
1931–1940	9.8 ± 2.8	3.5*	8.2 ± 2.5	3.3*
1916–1940	6.4 ± 3.0	2.1	4.1 ± 2.9	1.4

have only been partially taken into account. It would have been better probably to make the analysis in terms of person-years exposure to risk of death, but it would have been very difficult to do so with the type of record and equipment at our disposal.

Another criterion which might be used in the evaluation of the different methods of care and treatment of the tuberculous patient is the proportion of cases subsequently readmitted to the Sanatorium. The percentage of readmissions in

this series of cases, classified according to treatment prior to first discharge from the Sanatorium and the stage of disease and condition of the patient at the time of that discharge, is given in table 8. The total frequencies in the various categories do not agree with those previously quoted. Six patients diagnosed as tuberculous on first discharge were subsequently found to be nontuberculous. Some of the patients who had no surgery prior to their first discharge were later readmitted for pneumothorax or some other form of collapse therapy. And a certain proportion of the cases with pneumothorax alone prior to their first discharge received some other form of surgical treatment after readmission to the Sanatorium.

TABLE 8

	NO SURGERY			PNEUMOTHORAX			OTHER SURGERY		
	Discharged	Readmitted		Discharged	Readmitted		Discharged	Readmitted	
	No.	No.	Per cent	No.	No.	Per cent	No.	No.	Per cent
Arrested									
Minimal + moderately advanced	735	148	20.1	147	26	17.7	85	23	27.1
Far advanced	189	60	31.7	154	34	22.1	155	41	26.5
Total	924	208	22.5	301	60	19.9	240	64	26.7
Not arrested									
Minimal + moderately advanced	826	242	29.3	159	62	39.0	118	54	45.8
Far advanced	811	297	36.6	180	78	43.3	244	110	45.1
Total	1,637	539	32.9	339	140	41.3	362	164	45.3
Total									
Minimal + moderately advanced	1,561	390	25.0	306	88	28.9	203	77	37.9
Far advanced	1,000	357	35.7	334	112	33.5	399	151	37.8
Total	2,561	747	29.2	640	200	31.2	602	228	37.9

As one would expect, those whose tuberculosis was not arrested at the time of first discharge from the Sanatorium were much more likely to need readmission for further treatment than those whose tuberculosis was arrested on discharge. This is true no matter what type of treatment the patient received prior to his discharge from the Sanatorium. All of the differences between the per cent readmissions from those arrested and those not arrested on discharge were statistically significant. In the "No Surgery" group there was a much larger percentage of readmissions among those whose tuberculosis was far advanced at the time of their first discharge. In the two groups which had collapse therapy prior to first discharge, there were practically no differences in the per cent readmitted

between those whose tuberculosis was far advanced at discharge and those discharged with minimal or moderately advanced tuberculosis.

In general there were no significant differences in percentage of readmissions between the group with no surgery and those with pneumothorax alone prior to first discharge. The group which had some other type of collapse therapy showed a somewhat greater percentage of readmissions than either of the other two groups. The differences are not large but they are statistically significant. All of the differences which are statistically significant are summarized in table 9.

On the whole the postsanatorium history of the collapse cases is somewhat better than that of the noncollapse cases, mainly because a larger proportion of the cases receiving some type of surgical treatment were discharged from the Sanatorium with their tuberculosis arrested.

The final criterion of the success of Sanatorium treatment of the tuberculous patient is his ability to adjust himself satisfactorily after his discharge from the

TABLE 9

	Diff. ± S.E.$_{Diff.}$	t
Not arrested—Arrested		
NS	10.4 ± 1.9	5.5*
Pn.	21.4 ± 3.7	5.8*
S	18.6 ± 4.0	4.7*
Far advanced—(Minimal + moderately advanced)		
NS	10.7 ± 1.8	5.9*
S—Pn. Total	6.7 ± 2.6	2.6*
S—NS Total	8.7 ± 2.1	4.1*

Sanatorium. Table 10 shows the status of patients traced in 1941, classified according to type of treatment and condition on last live discharge.

We have already seen that for the group as a whole there has been a larger percentage of deaths among those who had no collapse therapy prior to their last live discharge than among those who had collapse therapy. The percentage of deaths during the twenty-five year period covered by this study is approximately twice as large for the no surgery group as for those who had some form of collapse therapy prior to discharge. The ratio remains the same whether we consider only those whose tuberculosis was at least apparently arrested at the time of their last live discharge from the Sanatorium, or those discharged as quiescent, improved or unimproved.

Table 11 gives the results of the comparisons between the three groups for expatients still alive in 1941. The percentages in the several work capacity categories have been recalculated on the basis of the number alive in 1941. It seemed desirable to use this basis rather than the number discharged alive because the percentage of deaths differed so markedly for the three groups to be compared.

In general, a slightly larger proportion of the no surgery cases reported that they were able to work at least part time in 1941. There were, however, no statistically significant differences between the group having no surgery and that which had pneumothorax alone. A slightly smaller percentage of the patients who had some other type of surgical treatment reported that they were able to

TABLE 10

	NO SURGERY		PNEUMOTHORAX		OTHER SURGERY		TOTAL	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Arrested								
Dead	124	20.9	13	4.9	26	10.6	163	14.7
Alive								
Work capacity unknown	62	10.4	46	17.3	42	17.1	150	13.6
Unable to work	68	11.4	33	12.4	48	19.5	149	13.5
Able to work part time	32	5.4	15	5.6	19	7.7	66	6.0
Able to work full time	308	51.9	159	59.8	111	45.1	578	52.3
Total	594		266		246		1,106	
Not arrested								
Dead	752	70.1	80	35.4	127	35.4	959	57.8
Alive								
Work capacity unknown	46	4.3	32	14.2	51	14.2	129	7.8
Unable to work	89	8.3	42	18.6	66	18.4	197	11.9
Able to work part time	23	2.1	8	3.5	25	7.0	56	3.4
Able to work full time	163	15.2	64	28.3	90	25.1	317	19.1
Total	1,073		226		359		1,658	
Total traced in 1941								
Dead	876	52.5	93	18.9	153	25.3	1,122	40.6
Alive								
Work capacity unknown	108	6.5	78	15.9	93	15.4	279	10.1
Unable to work	157	9.4	75	15.2	114	18.8	346	12.5
Able to work part time	55	3.3	23	4.7	44	7.3	122	4.4
Able to work full time	471	28.3	223	45.3	201	33.2	895	32.4
Total	1,667		492		605		2,764	

work than in either of the other two groups. None of the differences between the two collapse therapy groups was significant.

Among the arrested cases there was a significantly smaller percentage of ex-patients able to carry on normal activities in the group which had some form of collapse therapy other than pneumothorax alone, than in the group which had no surgical treatment prior to discharge. The ratio of the difference between the percentages to its standard error was 2.8, and the odds against a chance occurrence of a difference of that magnitude are in the neighborhood of two hundred to

one. For those whose tuberculosis was not arrested at the time of their last discharge, the difference was of the same sign but not large enough in comparison with its standard error to be statistically significant. However, the difference between the two groups, eliminating condition on discharge as a factor, proved to be unquestionably significant.

$$\text{Diff./S.E.}_{\text{Diff.}} = 12.3/3.8 = 3.2 \quad P = 0.0012$$

On the whole it seems reasonably clear that the chances of survival after discharge are considerably better for those who have had collapse therapy prior to discharge. But apparently such differences as exist in the proportion of cases able to adjust themselves and carry on a normal life as long as they do survive after discharge are in favor of the group which had no surgical treatment, no

TABLE 11

CONDITION ON DISCHARGE	WORK CAPACITY	TYPE OF TREATMENT					
		No Surgery		Pneumothorax		Other Surgery	
		Number	Per cent	Number	Per cent	Number	Per cent
Arrested	Unknown	62	13.2	46	18.2	42	19.1
	Unable to work	68	14.5	33	13.0	48	21.8
	Able to work	340	72.3	174	68.8	130	59.1
	Total	470		253		220	
Not arrested	Unknown	46	14.3	32	21.9	51	22.0
	Unable to work	89	27.7	42	28.8	66	28.4
	Able to work	186	58.0	72	49.3	115	49.6
	Total	321		146		232	
Total	Unknown	108	13.7	78	19.5	93	20.6
	Unable to work	157	19.8	75	18.9	114	25.2
	Able to work	526	66.5	246	61.6	245	54.2
	Total	791		399		452	

matter what the condition of the patient at the time of his last discharge alive from the Sanatorium.

SUMMARY

This is a follow-up study of 3,797 tuberculous patients with at least one discharge alive from Glen Lake Sanatorium during the twenty-five year period from 1916 through 1940. Of these, 2,764 were traced in 1941. The records were classified according to the type of treatment the patient received while in residence at the Sanatorium, bed-rest alone or in conjunction with some form of collapse therapy. Our purpose was to determine the effect of collapse therapy prior to discharge on the postsanatorium progress of the patient.

Although there was a smaller proportion of minimal and moderately advanced cases among those who had collapse therapy while at the Sanatorium, a larger

proportion of this group was discharged with tuberculosis arrested than in the group which had no surgery prior to discharge. Judging by past experience, the patients with collapse should on the average have a somewhat better postsanatorium prognosis than those without collapse.

The evidence seems to indicate that, no matter what the stage of disease or condition of the patient at the time of his last live discharge, his chances of survival are greater with collapse therapy than if he had no surgery prior to his discharge. The differences in the death rates in favor of the collapse groups are even more marked for those whose tuberculosis was in the far advanced stage and those in whom the tuberculous process was still active at the time they left the Sanatorium. There were, however, no real differences between the collapse and noncollapse groups in the actual number of years elapsing between date of discharge and date of death.

There were no significant differences in percentage of cases readmitted to the Sanatorium for further treatment from the noncollapse group and from those having pneumothorax alone. Nor were there any real differences between these two groups in their ability to carry on a normal existence after discharge from the Sanatorium. The postsanatorium progress of those patients who needed more drastic surgical treatment than pneumothorax alone was not quite so good as that of the cases having no surgery. True, the percentage of deaths was significantly less, but there was a greater percentage needing further sanatorium treatment and a smaller percentage able to carry on normal activity after discharge than among the noncollapse group.

SUMARIO

Trátase aquí de la observación subsecuente de 3,797 tuberculosos dados de alta por lo menos una vez, y en vida, del Sanatorio de Glen Lake en el período de 25 años transcurridos de 1916 a 1940 inclusive. De ellos se descubrió el paradero de 2,764 en 1941. Los protocolos fueron clasificados conforme a la clase de tratamiento que el enfermo recibió mientras residía en el sanatorio, ya fuera reposo en cama aislado o unido a algunas formas de colapsoterapia, abrigándose el propósito de determinar el efecto ejercido por la última antes del alta sobre la evolución postsanatorial del enfermo.

Aunque hubo una proporción más pequeña de casos mínimos o moderadamente avanzados entre los que recibieron la colapsoterapia mientras se encontraban en el sanatorio, también se dió de alta en un estado de estacionamiento a una proporción mayor de este grupo que en el grupo en que no se había aplicado cirugía antes del alta. A juzgar por la experiencia, los enfermos que han recibido la colapsoterapia deben en conjunto merecer un pronóstico postsanatorial algo mejor que los otros.

Los datos disponibles parecen indicar que, independientemente del período de la enfermedad o del estado del enfermo al darlo de alta por última vez, sus probabilidades de supervivencia son mayores cuando reciben la colapsoterapia que si no son tratados quirúrgicamente antes del alta. Las diferencias en la mortalidad en favor de los grupos que recibieron la colapsoterapia son aun más

decididas cuando la tuberculosis se encuentra en el período muy avanzado o en los enfermos en que el proceso tuberculoso continúa en actividad al abandonar el sanatorio. Sin embargo, no hubo verdaderas diferencias entre los grupos con colapso y sin él en lo tocante al número de años que transcurrieron entre la fecha del alta y la de la muerte.

Tampoco hubo diferencias significativas en el porcentaje de los casos readmitidos al sanatorio para tratamiento ulterior entre los grupos sin colapso y los tratados con neumotórax solo. Tampoco hubo mayor diferencia entre los dos grupos en su capacidad para llevar a cabo una vida normal después del alta del sanatorio. La evolución postsanatorial de los enfermos que necesitaban tratamiento quirúrgico más severo que el neumotórax solo, no es tán buena como la de aquéllos en que no se aplicó cirugía. Cierto es que el porcentaje de muertes es significativamente menor, pero también hubo un porcentaje mayor que necesitaron nuevo tratamiento sanatorial y un porcentaje menor capaz de llevar a cabo sus actividades normales después del alta que en el grupo sin colapso.

BIBLIOGRAPHY

(1) Aycock, G. F., and Keller, P. E.: Voluntary termination of artificial pneumothorax, Am. Rev. Tuberc., 1941, *45*, 117.

(2) Cold, Sigurd: Sammenligende undersøgelser over varighedsresultaterne ved konservativ behandling og pneumothorax behandling af kavernøs ftise, Nord. med. (Hospitalstidende), 1940, *8*, 1715.

(3) Dempsey, Mary: Statistical data, Am. Rev. Tuberc., 1942, *46*, 210.

(4) Epstein, H., and Hetherington, H. W.: Problems in the treatment of advanced tuberculosis in a municipal hospital, J.A.M.A., 1940, *114*, 723.

(5) Greentree, Leonard: Ambulatory pneumothorax: Its rôle in a program of treatment and prevention of tuberculosis, Am. Rev. Tuberc., 1941, *45*. 499.

(6) Hastings, D. R., and Behn, Borghild: Pneumothorax cases treated at a clinic, Am. Rev. Tubêrc., 1942, *46*, 656.

(7) Hurst, Allan, and Schwartz, Solomon: Pneumothorax treatment of pulmonary tuberculosis: Results in a follow-up study of 117 cases, Am. Rev. Tuberc., 1941, *45*, 132.

(8) Kellerman, F.: Effect of war conditions on a group of pneumothorax treated patients, Tubercle, 1941, *22*, 212.

(9) Morriss, W. H.: Pneumothorax treatment: The subsequent history of tuberculous patients after their selection for pneumothorax, Am. Rev. Tuberc., 1942, *46*, 629.

(10) Soderstrom, K. M.: Collapse therapy in moderately advanced tuberculosis: An inquiry into case selection and a statistical study of end results, Am. Rev. Tuberc., 1940, *44*, 173.

(11) Tice, F.: Ambulatory pneumothorax: Results of ten years' experience, Am. Rev. Tuberc., 1942, *46*, 639.

TUBERCULOMA OF THE POSTERIOR MEDIASTINUM[1]

BRIAN BLADES[2] AND DAVID J. DUGAN[3]

The diversiform nature of tuberculosis makes it necessary to include the disease in the differential diagnosis of a chronic obscure lesion in almost any part of the body. Thoracic disease and tuberculosis are so closely associated in the minds of both laymen and the medical profession that chronic pulmonary disease is apt to be explained by a perfunctory diagnosis of tuberculosis. There is no question that until recent years many patients with bronchiectasis, bronchogenic carcinoma and other chronic pulmonary diseases have been incarcerated in tuberculosis sanatoria to spend their remaining days taking the rest-cure.

Before surgical treatment of pulmonary disease was feasible, therapeutic endeavors were truly static—they consisted of rest. Differentiation between far advanced cases of tuberculosis and bronchiectasis or bronchogenic carcinoma was mainly of academic interest. The situation has changed considerably: Modern students of thoracic disease abandon the diagnosis of pulmonary tuberculosis quickly if roentgenological, clinical and laboratory evidence do not justify it. If accumulated data suggest that the lesion is a tumor, every effort must be made to establish the exact nature of the neoplasm. Not infrequently when dealing with intrathoracic tumors all ordinary methods will fail to establish a positive diganosis. In these instances it may be necessary to resort to exploratory thoracotomy. It is important to emphasize that thoracic exploration is the safest course to follow in suspected cases of intrathoracic neoplasm. If one waits for marked signs and symptoms of pain, enlargement of the tumor, etc., these manifestations will usually signify inoperability, and the opportunity for successful surgical excision of the lesion will have been lost. Moreover, the risk of open thoracotomy is trivial compared to the dangers of untreated thoracic tumors. It is safe to predict that the risk of a properly performed thoracotomy in a patient in good general condition is comparable to the hazard of an exploratory laparotomy.

It is inevitable, however, as more and more open thoracotomies are performed to identify intrathoracic masses of unknown origin, that in a small percentage of cases inflammatory lesions will be mistaken for tumors and will be excised. Graham and Singer (1) have described 2 examples of what they termed "calcified laminated abscesses" which were considered to be pulmonary neoplasms until after their removal. Haight (2) has reported a case of tuberculoma of the lung. The true nature of the tumor could not be established until the diseased portion of the lung was resected. Rendich and Camiel (5) have called attention to examples of massive conglomerate lesions of silicosis producing the roentgen appearance of pulmonary tumors. Recently, in the records from the Massa-

[1] From the Thoracic Surgery Section, Walter Reed General Hospital, Washington, D. C.
[2] Major, M.C., U. S. Army.
[3] Captain, M. C., U. S. Army.

chusetts General Hospital (3), a case was described in which total pneumonectomy was performed to remove a mass in the lung which was thought to be bronchogenic carcinoma. Microscopical examination of the tissue revealed a tuberculoma. Freelander and Wolpaw (4) have encountered 4 cases in which clinical and roentgenological findings of bronchogenic carcinoma were simulated by various inflammatory diseases of the lung. There are probably a good many instances of excision of inflammatory masses which have been mistaken for tumors which have not been recorded in the literature.

The cases which have been subjected to operations represent acknowledged errors in diagnosis. However, the principle of early removal of tumor masses when ordinary diagnostic methods fail to establish the true nature of the lesion is sound. In all probability the inflammatory masses would cause trouble later. The important fact, however, is that these cases represent the exceptions which prove the rule. The great majority of intrapulmonic masses demonstrable on X-ray films, which cannot be identified by ordinary methods, will prove to be malignant or potentially malignant tumors and should be extirpated.

The problem of distinguishing inflammatory from neoplastic disease in the mediastinum is less difficult than when the lungs are involved. Tuberculous lymph nodes, particularly in children, may suggest a tumor on X-ray examination but in most instances their location at the hilum of the lung and rather characteristic configuration will establish the diagnosis. The greatest chance for confusion would be mistaking tuberculous nodes for a tumor of lymphatic origin. Since the lymphomata are not often treated surgically and will respond to roentgen therapy the question of surgical exploration will not ordinarily be considered. A posterior mediastinal mass suggests a neoplasm of neurogenic origin or a chondroma or some of the rare types of mediastinal tumors might be considered. The possibility of tuberculosis producing a tumor-like lesion in the posterior-inferior mediastinum is remote. Grace (10) has reported a case of tuberculoma of the mediastinum which was considered to be a neoplasm until it was removed. The diagnosis was based on the microscopic appearance of the tissue. Tubercle bacilli were not found.

The case which we shall present was studied carefully; every modern facility for diagnosis was available and utilized. The true nature of the tumor was not suspected or even considered, however, until the mass from the mediastinum was removed.

CASE REPORT

The patient was a fifty-six year old white Army officer. About a year before the first admission to the Walter Reed General Hospital, he sought medical attention elsewhere because he had noted slight dyspnoea on exertion. He was advised to reduce the use of tobacco. No organic disease was detected but the possibility of bronchiectasis was considered. Bronchograms eliminated this possibility. A year later he complained of a dull aching sensation in the chest and was reëxamined. Roentgenograms of the chest at that time revealed a retrocardial mass (figure 1).

The patient was well nourished and well developed. The thorax was slightly emphysematous in contour. Tactile and vocal fremitus was increased slightly on both sides of the chest posteriorly. The physical examination was otherwise not unusual.

Examinations of the blood, including differential studies of the cells, were normal. Sputum cultures and smears did not contribute to the diagnosis. Urine examination revealed normal findings. Roentgenographic evidence, including conventional roentgenograms, fluoroscopy and X-ray films prepared with the Potter-Bucky technique,

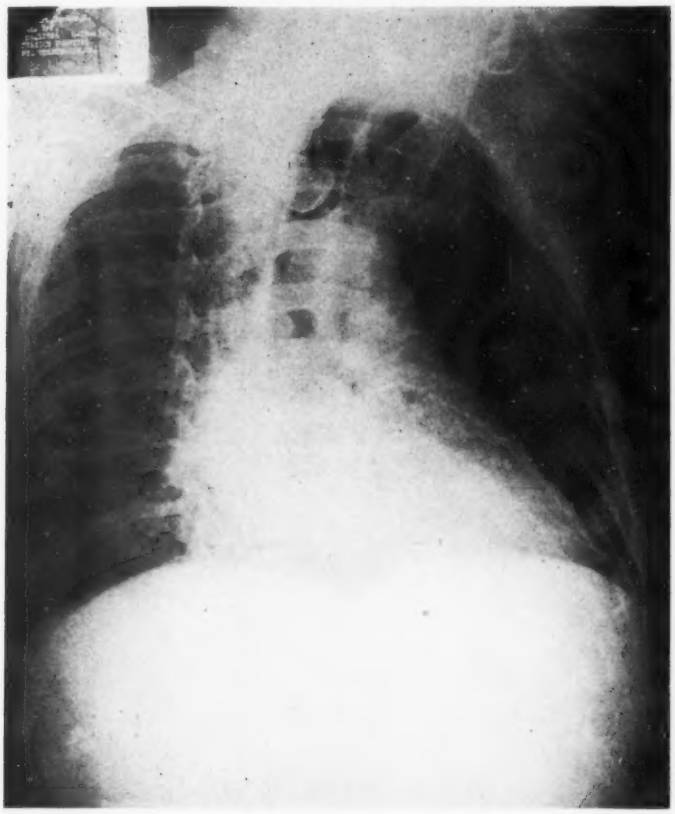


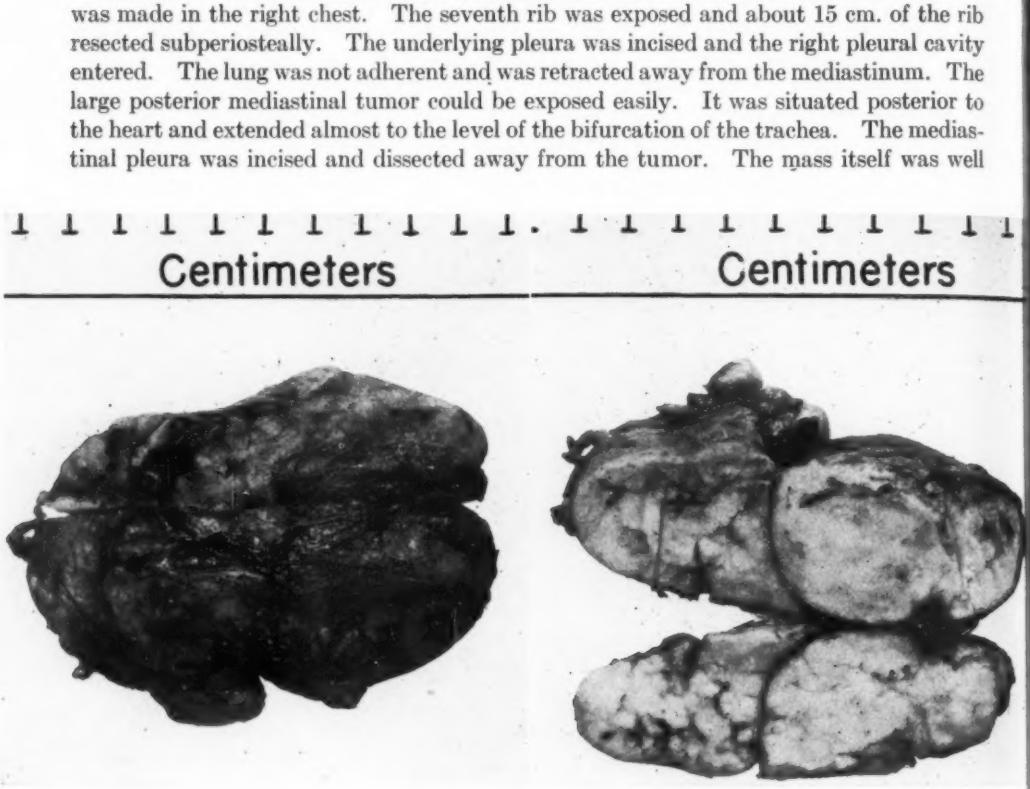
FIG. 1. Oblique roentgenogram showing tumor in the posterior mediastinum, in a retrocardiac position.

confirmed the impression that there was a mass in the posterior mediastinum in a retrocardiac position. The possibility of aneurysm was considered but visualization of the descending aorta with diodrast proved that the vessel was normal. The posterior position of the tumor and all of the clinical features suggested that the lesion was a primary nerve tumor of the posterior mediastinum.

It was necessary for the patient to leave the hospital for various reasons not related to the disease, but he was instructed to return in three months for reëxamination. Approximately three months later there was no change in the officer's condition, but roentgeno-

grams suggested that perhaps the tumor had increased in size. It was recommended at this time that an exploratory thoracotomy be performed and if possible the tumor be removed. The diagnosis remained posterior mediastinal tumor, type and cause undetermined.

Operation: Under intrathracheal cyclopropane anaesthesia, a postero-lateral incision was made in the right chest. The seventh rib was exposed and about 15 cm. of the rib resected subperiosteally. The underlying pleura was incised and the right pleural cavity entered. The lung was not adherent and was retracted away from the mediastinum. The large posterior mediastinal tumor could be exposed easily. It was situated posterior to the heart and extended almost to the level of the bifurcation of the trachea. The mediastinal pleura was incised and dissected away from the tumor. The mass itself was well



A B

FIG. 2A. Appearance of surface of tumor after removal
FIG. 2B. Cut section of tumor

encapsulated and was removed without difficulty. On the medial aspect, the tumor crossed the midline and extended into the left side of the posterior mediastimum. It was necessary to remove this portion of the tumor by blunt dissection. After the tumor was completely extirpated, there was no bleeding from the mediastinum. The mediastinal pleura was not closed over the bed of the tumor. The right lung was gently reinflated through the intrathracheal catheter and the chest wall was reconstructed in layers with fine silk. The wound was closed without drainage.

The mass was firm and had a grayish-white, meaty appearance. There were no zones of necrosis or caseation. On cut section an occasional anthracotic streak could be seen in the tissue (figure 2A and 2B).

The description of the microscopic section of the tissue by Captain W. S. Randall is as follows:

Sections reveal a lymph node throughout which there is considerable lymphocytic

hyperplasia with almost complete loss of normal follicular arrangement. Throughout are large discrete and conglomerate epithelioid tubercles, some showing early central caseation. In some of the tubercles this central caseous area is surrounded by radially arranged elongated cells. Many typical large giant cells are present throughout these tubercles, both in the central caseous areas and the periphery. Some of these tubercles are surrounded by distinct separate zones of lymphocytes and plasma cells (figure 3). In one section there is a focal zone of eosinophilia associated with an epithelioid reaction and many large multinucleated bizarre cells, the latter closely resembling those seen in Hodgkin's disease. Special staining reveals a rare acid-fast organism, morphologically representing tubercle bacillus.

The postoperative course was entirely satisfactory. It was necessary to aspirate straw colored fluid from the right pleura on two occasions. The fluid was sterile and did not con-

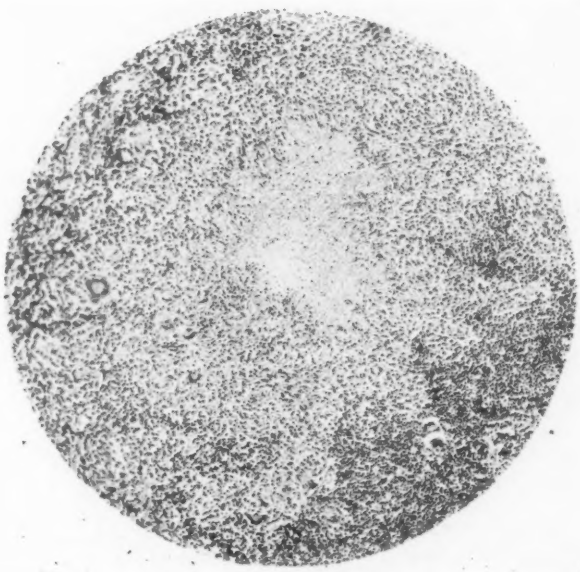


FIG. 3. Microscopic section of the tissue revealing tubercle formation

tain tubercle bacilli. The wound healed by primary union and the officer was discharged from the hospital twenty-one days after the operation. He is now on full military duty.

Comment: The finding of tuberculosis in this case came as a complete surprise to all the physicians who had examined the patient. The possibility had not been considered. When the true nature of the lesion was finally discovered, there was naturally some concern that a tuberculous effusion might result from the excision of the large tuberculoma.

Jacobaeus and Key (6) have reported a case in which a block resection of a tumor situated on the surface of the upper lobe and in the interlobar tissue resulted in death from the formation of a tuberculous abscess in the wound and tuberculous pleurisy. The roentgenological examination before the operation revealed a sharply outlined shadow without demonstrable reaction in the

surrounding pulmonary parenchyma. It was impossible to establish the diagnosis of tuberculosis until the tumor was excised. It is important to record that the authors mention that during the extirpation of the tumor there was "a discharge of matter" from the divided bronchi. Haight (2) has advocated the technique of block resection instead of enucleation when dealing with lesions which might be tuberculous because of the possibility of there being active tubercles at the periphery of the mass. It would seem that the principle of block resection with a margin of healthy tissue should be followed when any tumor of unknown origin is excised for identification.

The clinical features and roentgenological manifestations of active tuberculosis will usually give the surgeon fair warning, but it may be impossible to differentiate an old, retrogressive, sclerotic tuberculous lesion from a true neoplasm. There is comforting experimental evidence, however, to indicate that the excision of an encapsulated tuberculous mass will rarely result in dissemination of the disease. Griffith (7) has shown that the life of tubercle bacilli in a retrogressive lesion is brief and that old encapsulated lesions rarely contain live bacilli. Feldman and Baggenstoss (8) concur in this opinion and believe it is unusual to find virulent tubercle bacilli in encapsulated sclerotic lymph nodes. Their observations were based on a study of Ghon tubercles and tracheobronchial lymph nodes obtained at necropsy from 68 unembalmed bodies.

Jacobaeus and Key's case appears to be the only one in which wound infection and extension of the disease is reported following the removal of a tuberculoma because of an erroneous diagnosis of tumor. In other similar cases this complication has apparently not been encountered. Churchill's (9) dramatic success with 6 consecutive cases of lobectomy for tuberculosis, although not strictly comparable to the problem of so-called tuberculomata, adds further proof that properly performed extirpation of a tuberculous lesion in selected cases is not often complicated by dissemination of the disease.

CONCLUSION

An error in diagnosis is recorded which resulted in the removal of a tuberculoma of the posterior mediastinum. The mass was situated in a retrocardiac position and the preoperative diagnosis was primary nerve tumor of the posterior mediastinum. Evidence is presented which indicates that the removal of a well encapsulated tuberculous lesion usually will not cause dissemination of the disease. The dangers to which a patient is exposed from mistaking a tuberculoma for a tumor and removing it are insignificant when compared with the consequences of the appeasement of the "careful watching policy" when dealing with intrathoracic tumors.

CONCLUSIONES

Descríbese un error de diagnóstico que condujo a la extirpación de un tuberculoma de la porción posterior del mediastino. El tumor hallábase situado en una posición retrocardíaca y el diagnóstico preoperatorio fué neuroma primario de dicha región. Preséntanse datos indicativos de que la extirpación de una

lesión tuberculosa bien encapsulada por lo general no provocará diseminación del mal. Los peligros que corre un enfermo cuando se confunde un tuberculoma con otro tumor y se le extirpa son insignificantes, comparados con las consecuencias de la contemporización cuando se trata de tumores intratorácicos.

REFERENCES

(1) Graham, E. A., and Singer, J. J.: Three cases of resection of calcified pulmonary abscess simulating tumor, J. Thoracic Surg., 1936, *6*, 173.

(2) Haight, Cameron, and Farris, J. M.: Tuberculoma of the lung, J. Thoracic Surg., 1939, *9*, 109.

(3) Case Records of The Massachusetts General Hospital, August, 1943, *229*, 296.

(4) Freelander, S. O., and Wolpaw, S. E.: Chronic inflammatory lesions of the lung simulating bronchiogenic carcinoma, J. Thoracic Surg., 1940, *9*, 530.

(5) Rendich, R. A., and Camiel, M. R.: Massive conglomerate lesions of silicosis differentiated from pulmonary neoplasm, J. Thoracic Surg., 1943, *12*, 686.

(6) Jacobaeus, H. C., and Key, Einar: Some experiences with intrathoracic tumors, Acta chir. Scandinav., 1921, *53*, 600.

(7) Griffith, A. S.: Types of tubercle bacilli in human tuberculosis, J. Path. & Bact., 1929, *32*, 813.

(8) Feldman, W. H., and Baggenstoss, A. H.: The residual infectivity of the primary complex of tuberculosis, Am. J. Path., 1938, *14*, 473.

(9) Churchill, E. D., and Klopstock, Robert: Lobectomy for pulmonary tuberculosis, Ann. Surg., 1943, *117*, 641.

(10) Grace, A. J.: Tuberculoma of the mediastinum, J. Thoracic Surg., 1942, *12*, 131.

LOEFFLER'S SYNDROME[1]

Transient Pulmonary Infiltrations with Blood Eosinophilia

H. B. PIRKLE AND JULIA R. DAVIN

In 1931 Loeffler (1) first described transient pulmonary infiltrations with blood eosinophilia. The syndrome consists of asthma with cough, the occurrence of eosinophilia ranging from 10 to 60 per cent, the presence of a low-grade fever accompanied by mild leucocytosis and an elevated sedimentation rate. Chest signs are few or absent. The X-ray findings are the striking feature of the syndrome. They consist of pulmonary infiltrations in various parts of the lungs, usually in the lower fields. They disappear rapidly and do not cavitate. Loeffler did not stress the possible allergic nature of the condition, but subsequent observers have practically all suggested and sometimes demonstrated that the pulmonary infiltrations are on the basis of an underlying allergy (2, 3, 4, 5, 6, 7, 8). The subject is well reviewed by Hoff and Hicks in the AMERICAN REVIEW OF TUBERCULOSIS, February, 1942.

The case here reported is of interest to the general profession because the pulmonary infiltrations so strongly resembled pulmonary tuberculosis that a competent roentgenologist mistakenly diagnosed the case as one of tuberculosis.

CASE REPORT

B. A. B., white, housewife, aged fifty-four, was admitted to the Indiana State Sanatorium September 25, 1942. She had been well until the previous spring when she began to feel very tired and to cough slightly. A chest X-ray (figure 1) was taken August 5, 1942. It showed infiltration in the central and lower zones of the right lung with a small right-sided pleural effusion and infiltration in the left lower zone. A diagnosis of pulmonary tuberculosis was made and the patient placed on bed-rest at home pending admission to a sanatorium. Her temperature ranged from 99.2 to 101°F. and she had

FIG. 1. (Upper left.) Roentgenogram taken August 5, 1942. Infiltration in the central and lower zones of right lung; small pleural effusion right base; slight infiltration left lower zone.

FIG. 2. (Upper right.) Roentgenogram taken September 25, 1942. Effusion still present in right; considerable clearing in right lung; increased infiltration in left central zone.

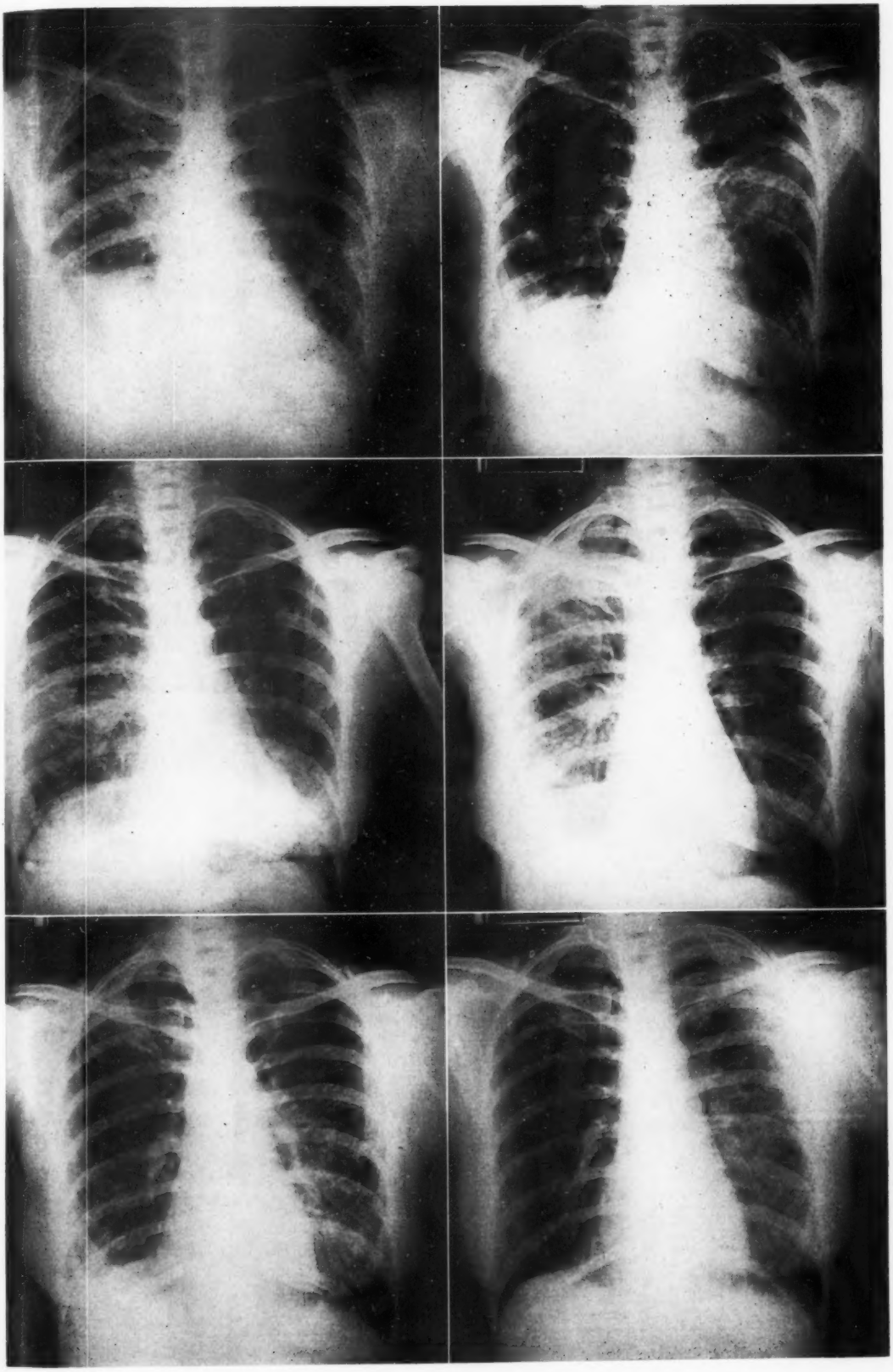
FIG. 3. (Center left.) Roentgenogram taken October 22, 1942. Disappearance of pleural effusion; increased infiltration right central and lower zones; considerable clearing in left.

FIG. 4. (Center right.) Roentgenogram taken November 17, 1942. Infiltration involving the whole right lung; left clear.

Fig. 5. (Lower left.) Roentgenogram taken December 8, 1942. Almost complete clearing in right lung; infiltration left central and lower zones.

FIG. 6. (Lower right.) Roentgenogram taken February 16, 1943. Right lung clear; new infiltration in upper half of the left lung.

[1] From the Indiana State Sanatorium, Rockville, Indiana.



FIGS. 1-6

a few night-sweats. A slight hacking cough was present. The symptom most annoying to the patient herself was the rapid pulse with palpitation. At no time was there any asthma.

At the time of this patient's admission to the Indiana State Sanatorium, September 25, 1942, the chest X-ray film (figure 2) showed a striking change from the plate of August 5, 1942. The effusion was still present on the right, but the infiltration in this side had cleared considerably, while a new area of mottling was present in the central zone of the left lung. Sputum studies, including gastric lavage and culture, were negative for tubercle bacilli. The blood picture showed some anaemia, 12.2 g. haemoglobin and 3,060,000 red blood cells. In the smear there were 15 per cent eosinophils. The total white count was 11,000. The sedimentation rate, Cutler's method, was 28 mm. in one hour. The patient did not appear very sick. Her temperature range was from 97.4 to 99.4°F. with a pulse range of 84 to 100. She complained chiefly of palpitation, nervousness and a sense of fatigue.

A search was begun for some aetiological factor. The stools were negative for parasites. The sputum continued negative for tubercle bacilli, yeasts, moulds, spirochetes and bacteria. A pyuria was found with many Gram-negative bacilli in the sediment of a catheterized specimen. Treatment with Sulamyd was started October 14, 1942, but was discontinued October 21, 1942, because of a temperature rise to 101.2°F.

An X-ray film (figure 3), taken October 22, 1942, showed another striking change. The pleural effusion had disappeared, there was now an infiltration in the lower two-thirds of the right lung and the left lung had cleared considerably. The white cell count on October 16 was 8,800 with 33 per cent eosinophils; on October 19, 12,150 with 27 per cent eosinophils. The temperature ranged from 98.2 to 100°F. for the next two weeks.

An X-ray film (figure 4), taken on November 17, 1942, revealed increased infiltration, now involving the whole right lung with the left lung practically normal. Eosinophilia on November 23 was 17 per cent with a total white cell count of 10,450. On December 9, 1942, Sulamyd was again started and again discontinued on December 13, 1942, because of a temperature rise to 100.4°F. An X-ray film (figure 5), taken on December 8, showed almost complete clearing in the right lung, with new infiltrations in the left central and lower zones. Eosinophilia on December 11 was 12 per cent with a total white cell count of 12,000. The sedimentation rate was 28.5 mm. in one hour.

The next chest X-ray film, taken on January 12, 1943, revealed both lungs practically clear. No blood count was made at this time. The temperature and pulse were settling to normal.

On February 1, 1943, a course of mandelic acid was given and proved effective. On February 14, 1943, a catheterized specimen of urine showed only an occasional pus cell.

The next X-ray film (figure 6), taken on February 16, 1943, showed a recurrence of infiltrations in the left lung. A month later, March 17, 1943, both lungs were nearly clear. There were still 13 per cent eosinophils in the blood smear with a total white cell count of 7,700. Finally, on May 4, 1943, an X-ray film showed the lungs completely clear. The eosinophilia had dropped to 5 per cent with a total white cell count of 8,200 and the sedimentation to 20 mm. in one hour. Clinically the patient was well, with a temperature range of 97.4 to 99.2°F. and a pulse range from 84 to 100.

Our last chest X-ray film, taken on June 10, 1943, showed a normal chest. The eosinophil count was 7 per cent, total white count 8,750. The patient was ambulant and well, except for a slightly rapid pulse and nervousness. It was decided to enter her at the Robert Long Hospital, Indianapolis, Indiana, for further stool examinations and basal metabolism determinations. These studies proved to be normal.

DISCUSSION

The patient presented here differs from certain of the other reported cases of Loeffler's syndrome in the absence of asthma and in the long duration of the pneumonic migrations. Karan and Singer (9) described 5 cases of transient pulmonary infiltrates misdiagnosed as tuberculosis. Their fifth case bears a strong resemblance to ours. The pneumonitis continued to migrate for three months; in our case, for eight months.

The aetiology of the syndrome remains obscure. Certainly no convincing aetiological factor was ever found in our case. This woman is now employed at the Indiana State Sanatorium as a nurse's aid. She continues to be very nervous with easy fatigability and a tendency to tachycardia, but chest X-ray films taken at intervals of two months remain clear and there is no eosinophilia.

SUMMARY

A case of transient pulmonary infiltrations associated with blood eosinophilia has been presented.

SUMARIO

En este estudio descríbese una observación de infiltraciones pulmonares transitorias asociadas con eosinofilia.

REFERENCES

(1) Loeffler, W.: (A) Zur Differential-Diagnose der Lungen Infiltrierungen; (B) Über flüchtige Succedan-Infiltrate mit Eosinophilie, Beitr. z. Klin. d. Tuberk., 1932, *79*, 338.

(2) Weber, F.: Transient pulmonary infiltrations with blood eosinophilia, Brit. J. Child. Dis., 1939, *36*, 15.

(3) Soderling, B.: Transient lung consolidation, Arch. Dis. Childhood, 1939, *14*, 22.

(4) Stefano, Jose: Sindrome asmatico por amebiasis, Semana méd., 1939, *46*, 749.

(5) Lavieri, G., Bariety, M., and Caroli, J.: Distomatose hepatique et syndrome de Loeffler, Paris méd., 1939, *1*, 434.

(6) Breton, A.: Apropos de la radiologie du syndrome de Loeffler, Paris méd., 1938, *1*, 538.

(7) Cohen, R.: Le syndrome de Loeffler, Presse méd., 1938, *46*, 797.

(8) Gravesen, P. B.: Transitory lung infiltrations with eosinophilia, Acta med. Scandinav., 1938, *96*, 523.

(9) Karan, A. A., and Singer, E.: Transitory pulmonary infiltrations mistaken for tuberculosis, Ann. Int. Med., 1942, *17*, 106.

CHEMOTHERAPY OF CLINICAL TUBERCULOSIS WITH PROMIN

P,P′-Diaminodiphenylsulfone-N,N′-Didextrose Sulfonate

A Second Report of Progress

H. CORWIN HINSHAW,[1] KARL H. PFUETZE[2] AND WILLIAM H. FELDMAN[3]

In May, 1942, we presented a preliminary report of progress before the meeting of the National Tuberculosis Association in Philadelphia, dealing with our experiences in the treatment of clinical tuberculosis with promin, with special reference to a group of 36 patients who had received what we considered to be adequate doses of the drug for from four to twelve months (1). At the present time (December, 1943), we wish to report further upon the clinical progress of these same patients during the time which has elapsed since this earlier report.

Our previous report divided the 36 "adequately treated" patients into four groups on the basis of the degree of improvement as demonstrated by objective criteria at that time. We shall now reconsider these same groups of patients and describe their present clinical status and the trend observed.

GROUP 4—"MARKED IMPROVEMENT"

Eight patients were classified in this group in the earlier paper. All had shown consistent, striking and unexpected improvement while receiving chemotherapy. Roentgenographic evidence of improvement was demonstrable in all cases. Sputum had been converted from positive to negative in 6 cases (75 per cent). The average sedimentation rate decreased from 29 mm. to 7 mm. in one hour (Cutler method). None of these patients had shown favorable clinical trends prior to treatment. In 5 of the cases the disease was in a far advanced stage of development, and in 3 cases the disease was moderately advanced.

At the present time 6 of these 8 patients have consistently negative sputum as determined by microscopical examination and repeated cultures of gastric contents. Clinical improvement has continued in all cases, at least while the patients were taking promin. One patient left the sanatorium against medical advice but in a markedly improved state, and subsequently suffered a slight extension of his disease. This patient reëntered the sanatorium, received further treatment, and the disease has again apparently come under control. Of the remaining 7 patients, 6 have the disease in a definitely arrested form and 5 of these have now undertaken employment. The only patient in the entire group who continues to have a consistently positive sputum is one who had a huge cavity, 4.5 by 6 cm. in size, together with considerable fresh tuberculosis at the time her treatment was instituted in July, 1941. Treatment was continued for slightly more than a year with rapid clearing of exudative lesions, but

[1] Division of Medicine, Mayo Clinic, Rochester, Minnesota.
[2] Cannon Falls, Minnesota.
[3] Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota.

she still has a large cavity with no other roentgenographic evidences of active disease. Her general condition has steadily deteriorated during the past year.

It must be emphasized that all patients in this group received relatively large doses of promin, well in excess of what the average patient will readily tolerate. This average dose was considerably larger during earlier phases of treatment, at a time when therapy appeared to be more urgently required. It seems possible that their unusually favorable clinical trends may have been related to their superior tolerance for promin. It should also be emphasized that the clinical trend before promin was given was definitely in an adverse direction for nearly all members of this group, but adequate trial of rest in bed had only been carried out in approximately half of these cases. This pretreatment "control" period averaged sixteen months in duration.

GROUP 3—"MODERATE IMPROVEMENT"

There were 6 patients in this group who were so classified because of reasonably satisfactory progress as demonstrated by measurable methods, but the rate and degree of improvement did not equal those observed in group 4.

With the exception of one patient who died after thoracoplasty, all patients in this group have progressively improved and now have arrested disease with negative sputum, including negative culture of gastric contents. In 2 cases thoracoplasty was necessary to attain this result. Three patients are employed.

A "control" period of rest in bed was carried out before institution of chemotherapy in only 3 of the 6 cases in this group with no significant improvement being noted during this period, which averaged eighteen months in duration.

New lesions have not developed in any of the cases in this group, nor has any increase of preëxisting lesions been observed at any time since institution of chemotherapy.

GROUP 2—"SLIGHT IMPROVEMENT"

Eight patients were assigned to this group because of slight improvement, although in no instance was this of unexpected degree. Two patients in this group have been dismissed from the sanatorium with their disease in an arrested state. In one of these, collapse therapy had been instituted and in the other the original lesion was not an extensive one. Four additional patients in this group have made excellent clinical progress during the past year, aided in 2 instances by thoracoplasty. All of these had most extensive destructive disease involving a large portion of both lungs. The 2 remaining patients in this group have made no progress and their condition appears to be slowly getting worse; the prognosis has appeared hopeless at all times.

GROUP 1—"NO IMPROVEMENT"

Despite chemotherapy the 8 patients in this group had shown no change in their clinical condition during our previously reported period of observation. Since the time of our previous report, one patient with a lesion of bronchostenotic type has improved sufficiently to be dismissed and has had negative sputa

only since March, 1942. Two additional patients have received surgical collapse and are progressing satisfactorily. The remaining 5 patients in this group have shown no significant change except for one who died of pulmonary haemorrhage.

Two patients reported in our previous paper were listed as having had extension of their disease. Chemotherapy was continued in both of these cases and the disease eventually appeared to be under control. In one of these cases the disease is now classified as "arrested" and the patient is working; the other achieved temporary arrest of her disease for eight months but subsequently suffered further extension when treatment was discontinued and under the stress of employment. She is again improving and receiving chemotherapy.

Four patients with extensive hopeless disease, treated during terminal stages were dead at the time of our previous report.

COMMENT

It is difficult to state with absolute conviction that chemotherapy was the crucial factor in the recovery of any of these patients from tuberculosis. However, subsequent events have clearly indicated that their previously reported improvement was genuine and the reversal of their unfavorable clinical trend was consistent. It seems probable to us that the sanatorium regimen contributed to their recovery, and in some instances thoracoplasty or other surgical collapse also was a necessary procedure. In some of these latter cases, chemotherapy may have aided in stabilizing the recent exudative tuberculosis, which originally involved the better lung, so that thoracoplasty could be carried out on the opposite side.

Chemotherapy has now been discontinued in 20 cases for periods varying between ten and twenty-five months (average nineteen months). Only 3 patients have suffered reactivation of tuberculosis following discontinuation of chemotherapy, and in 2 instances this may be due to the fact that they left the sanatorium contrary to medical advice. These 2 patients are again improving and receiving further chemotherapy.

A priori reasoning would not lead one to expect that any form of chemotherapy should bestow permanent benefit after discontinuation of treatment. Tuberculosis may constitute an exception to this statement in view of experimental results which have been reported. Feldman, Hinshaw and Moses (2) (1942) and Medlar and Sasano (3) (1943) have reported experiments which appear to demonstrate that guinea pigs may live for many months following successful chemotherapy for experimental tuberculosis, even though treatment has been discontinued and even though fully virulent tubercle bacilli could be demonstrated in their organs. In the earlier experiments reported (Feldman, Hinshaw and Moses), animals were permitted to live without treatment for eighty-two days, which was comparable to the time required for all control animals to succumb to tuberculosis. Even after this period, no lesions could be demonstrated, grossly or microscopically, in the organs of predilection of some animals despite the fact that fully virulent tubercle bacilli were found to be present when suspensions of the spleen were subsequently injected into guinea pigs. These results

were regarded as evidence that some form of immunity had developed in these previously treated animals which permitted them to harbor tubercle bacilli without developing disease. Medlar and Sasano (3) extended these observations over a period of eighteen months and demonstrated that this immunity may be overcome eventually. An extension and elaboration of this experiment now in progress in our laboratory is further confirming and extending Medlar's results.

Guinea pigs possess so little natural defense against tuberculosis that any demonstration of acquired resistance becomes of great interest. Human beings possess such marked natural resistance against tuberculosis that it would be difficult to assemble convincing evidence that any added resistance had been artificially supplied by any therapeutic effort. It is extremely important to emphasize that human beings, unlike guinea pigs, are usually in need of but little assistance in order to overcome a losing fight against tuberculosis. A small increment of help supplied at the proper time to a tuberculous lesion of reversible character might be adequate to reverse a progressive disease totally and permanently. There are no better demonstrations of these facts than in the field of collapse therapy. Any additional power which chemotherapy might add to the physician's therapeutic armamentarium against tuberculosis is clear gain because it may be used in combination with all previously known methods of treatment without conflict.

At no time during the course of these observations was it anticipated that they could be adequate to yield the type of evidence which will be required to place chemotherapy of tuberculosis upon a sound basis. However, they have served us a very useful purpose in demonstrating that prolonged administration of promin orally is clinically feasible and they have provided sufficiently suggestive evidence of therapeutic effect to make more extensive and more exactly controlled observations highly desirable. The study has shown some indication as to the types of patients most likely to respond to treatment and will be of value in planning our future investigations.

It would be well to point out some shortcomings of the observations recorded, in the hope that more conclusive studies may be made. The wide variety of types of lesions included in this study makes judgment nearly as difficult as if a variety of separate diseases were being considered. This fact and the small total number of participating patients result in fragmentary information which may be quite encouraging and stimulating to further study, but do not satisfy the rigid requirements which should be demanded before conclusions are drawn. It appeared impossible to find a comparable control group in our clinical material to compare adequately with the wide variety of lesions treated. An attempt was made to compare each patient's clinical course before and after institution of treatment, but this procedure could not exclude the factors of natural evolution of the disease. In most instances in which this procedure could be utilized, it served to indicate a therapeutic effect of the drug.

A review of the theoretical consideration and of the factual evidence makes it appear probable that reversible lesions of recent origin (exudative type), even

though extensive, are more likely to respond to chemotherapy than are lesions which are the result of destruction of tissue and efforts at repair (caseation, cavitation, fibrosis). It must be emphasized again that the need for accurate control studies is most urgent in observing effects upon lesions of this type because of their well known instability, which may lead to either spontaneous resolution or to progressive destructive types of tuberculosis. We have been greatly encouraged by the fact that none of our patients with such lesions who received chemotherapy have shown progressive trends and resolution has occurred in all cases. The number of cases, however, is small.

There are definitely undesirable toxic reactions to promin and to other drugs of the sulfone series. The limiting factor in dosage has usually been the production of a mild to moderate "haemolytic" anaemia, after prolonged administration. Fortunately, this is a reversible and a measurable effect; to a certain extent it is predictable. Furthermore, our observations have shown that anaemia of considerable degree does not appear to hinder the healing process in clinical tuberculosis. A few patients of this series made striking and consistent gains during a time when the haemoglobin content of their blood, as a result of chemotherapy, was constantly less than 10.0 g. per 100 cc.

A recent report (4) of potentially great significance described the use of promin in treatment of human leprosy and included the following statements: "It can be regarded as the most encouraging experimental treatment ever undertaken at the National Leprosarium. The writers are not in a position at this time to state that it possesses any specific action upon Hansen's bacillus. They consider it an advance in the right direction in the chemotherapy of leprosy and hope that further synthesis of the sulfa chemicals will produce a product which has specific properties against *M. leprae* and *M. tuberculosis* . . . .

"Promin can be considered to have opened a new avenue in the chemotherapy of the mycobacterial diseases. It is hoped that further synthesis of sulfa compounds may produce a substance which will succeed in saving countless lives in this still dark field of medicine."

SUMMARY

A group of 36 patients who first received chemotherapy for tuberculosis during 1941 were considered in a preliminary report in May, 1942. The present communication describes their clinical course up to December, 1943. The originally described clinical trends have been consistent and progressive, but the number of patients is not sufficiently great and the observations are not adequately controlled to permit unqualified conclusions regarding the value of promin as a therapeutic agent in the treatment of pulmonary tuberculosis. The results are sufficiently suggestive and encouraging to make it urgent that a series of large and fully controlled studies be undertaken to confirm or deny these preliminary impressions. Exudative lesions of recent origin appear to be more promising types for chemotherapy than those which involve caseation, necrosis, cavitation and fibrosis. The toxic manifestations of promin and related drugs are to be constantly reckoned with but are measurable, reversible and control-

lable and do not appear to have any adverse effect upon the clinical course of tuberculosis. Progress is continuing at a satisfactory rate toward development of a clinically practicable chemotherapeutic agent for use in selected cases of tuberculosis. We believe that a great deal of work remains before attainment of this goal.

SUMARIO

En un informe preliminar de mayo 1942, se estudió un grupo de 36 tuberculosos que fueron los primeros en recibir la quimioterapia durante el año 1941. La comunicación actual describe la evolución clínica de los mismos hasta diciembre, 1943. Las tendencias clínicas descritas primitivamente han sido constantes y cada vez más acentuadas, pero el número de enfermos no es suficiente ni las observaciones han sido comprobadas adecuadamente para poder sacar conclusiones irrefutables acerca del valor de la promina como elemento terapéutico en la tuberculosis pulmonar. Los resultados, sin embargo, son suficientemente indicativos y alentadores, y urge emprender una serie de estudios en gran escala y debidamente comprobados que conformen o refuten las impresiones preliminares. Las lesiones exudativas recientes parecen prometer más a la quimioterapia que aquellas en que existen caseación, esfacelo, cavernas y fibrosis. Hay que tomar constantemente en cuenta las manifestaciones tóxicas de la promina y las drogas afines, pero son mensurables, invertibles y cohibibles, y aparentemente no ejercen efecto nocivo alguno sobre la evolución clínica de la tuberculosis. Continúase adelantando satisfactoriamente en el sentido de obtener un elemento quimioterapéutico, clínicamente usable en casos seleccionados de tuberculosis, pero resta todavía mucho por hacer antes de alcanzar esa meta.

REFERENCES

(1) Hinshaw, H. C., Pfuetze, Karl, and Feldman, W. H.: Treatment of tuberculosis with promin: Progress report, Am. Rev. Tuberc., 1943, *47*, 26.

(2) Feldman, W. H., Hinshaw, H. C., and Moses, H. E.: Promin in experimental tuberculosis: Sodium p,p'-diaminodiphenylsulfone-N,N'-didextrose sulfonate, Am. Rev. Tuberc., 1942, *45*, 303.

(3) Medlar, E. M., and Sasano, K. T.: Promin in experimental tuberculosis in the guinea pig, Am. Rev. Tuberc., 1943, *47*, 618.

(4) Faget, G. H., Pogge, R. C., Johansen, F. A., Dinan, J. F., Prejean, B. M., and Eccles, C. G.: The promin treatment of leprosy. A progress report, U. S. Pub. Health Rep., November 26, 1943, *58*, 1729.

CULTIVATION OF THE BOVINE TUBERCLE BACILLUS[1]

ANDRES R. ARENA AND ABEL CETRANGOLO

The first seeding of bovine tuberculous material on ordinary solid culture medium, with rare exceptions, results in slow growths of small colonies which do not change greatly between thirty and forty-five days, even under the best conditions of humidity and temperature. The specimens of bacilli of Koch, which show this condition, have been termed "dysgonic" by Griffith (1). "Dysgonism" is characteristic of all freshly isolated bovine tubercle bacilli and on rare occasions is also observed in human strains. As will be observed later, it becomes of practical interest to recognize the opposite effects of glycerin on the growth of these two principal strains of tubercle bacilli. As a rule, 5 per cent glycerin on solid media entirely inhibits the development of the bovine bacillus while it augments the growth of the human bacillus.

The reaction of the bovine bacillus on liquid medium, however, is different, especially if the medium contains no glycerin. Valtis and Van Deinse (2) have called attention to the eugonism obtained by the bovine bacillus from both man and cattle when cultivated on the medium of Besredka, as evidenced by the change in morphology of the original colonies as well as by the transplants to solid media.

On the contrary, the growth of the human bacillus in the liquid medium of Besredka develops at first very slowly and only after a more or less extended period of transplantations will it become eugonic. In this respect it resembles the bovine strain in glycerinated media. But it is a well known fact that both the bovine bacillus on a solid glycerinated medium and the human bacillus on a liquid medium without glycerin may be converted into eugonic types after long intervals of time which vary with each strain.

Our experiences also have shown that the eugonism acquired by the bovine bacillus develops more quickly if the bacillus originates from man or another animal species than if it is isolated from bovine lesions (3). This phenomenon, which we have noted on various occasions, makes us believe that the morphological aspects of the cultures of the bovine bacillus become more unstable when passed through another animal species.

The difference in behavior of human and bovine bacilli in the medium of Besredka might seem to present a scientific basis for the initiation of the classification of any particular strain. But this idea has only a superficial meaning. The most important criterion for determining type is the study of the virulence, to accomplish which it is above all indispensable to verify the purity of the culture before inoculation into animals. When the culture is obtained from liquid culture media, the results have only an uncertain and relative value because they do not permit of a differentiation of tubercle bacilli from other acid-fast bacilli, nor from the non-acid-fast bacteria, which might exist as an accidental contami-

[1] From the Catedra de Patologia y Clinica de la Tuberculosis, Dr. Raul Vacarrezza, Director, Buenos Aires, Argentina.

nation resulting from improper purification. The verification of purity, therefore, must be completed by seeding on solid culture medium. Instead of simplifying, the technique is obviously rendered more complicated by the attempted use of liquid media for typing.

For these reasons we think it preferable to use the method of direct seeding of the purified material on solid culture medium with and without glycerin. The medium recommended by Jensen (4) for this purpose is that of Loewenstein (5), 1930, because of the small quantity of glycerin which it contains (0.75 per cent total volume). The diminution of glycerin undoubtedly facilitates the cultivation of the bovine bacillus and consequently this culture medium is indicated for that purpose. If by the use of such a medium alone it is intended to differentiate human from bovine bacilli found in tuberculous materials originating from man, we believe that in addition to the difficulty of the task we would expose ourselves to the possibility of grave errors.

In 1937 we (6) made an investigation under the direction of Professor Sayago in the Institute of Phthisiology in Cordoba (Argentina) on the action of glycerin on the cultures of bacilli of Koch originating from man. Since then we have confirmed those results and made other observations in the laboratories here. In addition, we studied the use of various media for isolation of the bovine type irrespective of the origin, and wish to report our several findings.

The culture media used were those of Loewenstein, with and without glycerin, prepared in accordance with that author's formula (7). The material of the investigation was sputum treated with 5 per cent sulphuric acid for twenty minutes at 38°C. The sediment was washed repeatedly and passed through filter paper according to the technique used by us on another occasion (6). After filtration we sowed the same number of drops of the filtered liquid on each tube of the culture medium and injected rabbits intrapleurally with 0.01 mg. wet weight of the growth from the first sowing. The technique of inoculation corresponded to that of Boquet and Laporte (8), which technique we believe we have simplified by using a simple manometer which avoids the incision advised by the authors.

The results are to be seen in table 1 and show that the human bacilli in media without glycerin generally appear in small, semi-spheric, whitish colonies of smooth surface and correspond to the appearance of colonies of bovine bacilli; that the same material sown in the glycerinated medium in the majority of cases resulted in eugonic cultures either with rugose, mulberry-form, thread-like or cerebriform colonies; and that apparently the glycerin determines the eugonism of the human type of bacillus as well as influences the form and consistency of the colonies, but that it had no effect on the virulence of initial cultures because the effect on the rabbit was practically the same for bacilli when grown on the medium of Loewenstein, with or without glycerin.

All the results were by no means typical. There was an atypical culture (No. 4) which grew into smooth dysgonic colonies without glycerin and rough and eugonic colonies on glycerinated medium, but the virulence tests alike in both corresponded to the bovine type. Aside from these more striking reactions there

TABLE 1

RESULTS OF SOWING						RESULTS OF INOCULATION WITH RABBITS	
Number	Date of sowing	Patient	Dates of observation	Loewenstein without glycerin	Loewenstein with glycerin	With glycerin	Without glycerin
1	8-28-37	L. L.	9-13-37	l	l	Killed after 110 days; lesions corresponding to human bacillus.	Died after 77 days with caseous pleural pulmonary lesions of inoculated side.
			10- 7-37	L	R		Died after 53 days; nodules in pericardium and lungs.
2	8-27-37	N. B.	9-13-37	l	rR	Killed after 143 days; lesions of human type.	Killed after 143 days; lesions of human type.
			7-10-37	L	rR		
3	8-27-37	I. N.	9-13-37	l	l	Died after 50 days; lesions in pleura and lung of inoculated side.	Died after 57 days pulmonary, pleural and and pericardial lesions.
			10- 8-38	L	M	Killed after 102 days; caseous lesions in lungs, nodules in pleura and pericardium.	Killed after 102 days; lesions of human type.
4	8-27-37	E. A.	9-13-37			Died after 78 days; many nodules in pericardium and pleura, caseous lesions in lung of inoculated side, nodules in kidney and liver.	Died after 49 days; lesions in pericardium, pleura and lungs.
			8-10-37	L	R	Killed after 97 days; caseous lesions in both lungs nodules in pleura, pericardium and liver.	Killed after 97 days; lesions correspond to lesions caused by bovine bacillus.
5	8-28-37	F. C.	9-13-37	l	l	Died after 67 days; lodules in pleura, pericardium, lungs and liver.	Died after 47 days; tuberculous pericarditis.
				m	M	Killed after 96 days; nodules in pericardium and pleura and caseous lesions in lung	Killed after 96 days; lesions less marked than from medium without glycerin.
6	9- 1-37	J. M.	10- 8-37	l	M	Killed after 99 days; small nodules in percardium and pleura, abundant in the lung.	Killed after 99 days; lesions less than in the preceding.
7	9- 2-37	A. Ch.	10- 8-37	l	M	Killed after 100 days; lesions of human type.	Killed after 100 days; lesions of human type.
8	9- 7-37	H. T.	10- 8-37	l, c	M	Killed after 114 days; abundant caseous nodules in pericardium and lungs.	Killed after 114 days; similar to other.
9	9- 9-37	J. B.	10- 8-37	l, c	L	Killed after 114 days, lesions of human type.	Killed after 114 days, lesions of human type.
10	9-24-37	O. Z.	11- 2-37	L	Cerebroid granulated	Killed after 94 days; lesions of human type.	Killed after 94 days; lesions more pronounced. Caseous nodules in lung.

TABLE 1—*Concluded*

RESULTS OF SOWING						RESULTS OF INOCULATION WITH RABBITS	
Number	Date of sowing	Patient	Dates of observation	Loewenstein without glycerin	Loewenstein with glycerin	With glycerin	Without glycerin
11	9-24-37	B. F.	11- 2-37	l	Threads and l	Killed after 98 days; lesions of human type.	Killed after 98 days; lesions similar, but more pronounced caseous nodules in lung.
12	10-13-37	B. O.	11-14-37	l	R	Killed after 84 days; lesions of human type.	Killed after 84 days; lesions of human type.
13	10-18-37	S. C.	11-17-37	l	R	Killed after 86 days; nodules in pleura and pericardium, caseous nodules in lung.	Killed after 86 days; the same as without glycerin.

l, Smooth, semi-spheric colonies of less than ½ mm. of diameter.
L, Smooth, semi-spheric colonies of more than 1 mm. of diameter.
c, Flat colonies approximately 1 mm. diameter.
r, Rugose colonies less than ½ mm. diameter.
R, Rugose colonies more than 1 mm. diameter.
m, Colonies in form of mulberry, of less than 1 mm. diameter.
M, Colonies in form of mulberry, of more than 1 mm. diameter.

were less definite ones, namely, out of the 13 specimens examined there were 6 human strains in which the virulence for the rabbit injected intrapleurally is greater than that usually assigned to the bacillus of the human type; the human bacillus sometimes becomes eugonic in the glycerin-free liquid medium of Besredka; but if sown from the beginning on the medium of Loewenstein without glycerin and transplanted for more than a year on the same, it remains dysgonic.

Finally, by comparing the result of the cultures of cases 3, 4, 5, 9 and 11 with that of the inoculations, it is obvious that the morphological difference of the colonies developed on the medium of Loewenstein, with or without glycerin, does not permit of a final statement with regard to the virulence of the specimens.

For all these reasons we have come to the conclusion that culturing alone on the prevailing media, although indispensable for the determination of the types of tubercle bacilli, has only a relative value and must be supplemented by that of inoculation, more particularly if the material originates from man.

It seemed desirable to explore the field further and attempt to find a more suitable medium for the isolation of all strains irrespective of the source.

As far as the culture medium for bovine bacilli is concerned, we tried to obtain not only a medium which may offer, in the first sowing of material originating from tuberculous cattle, the greatest quantity for the purpose of facilitating the weighings, but also one which might serve for the preservation of the specimens without the alterations imposed on them by the use of glycerinated media. We also wished to accomplish the purposes which the Argentine Committee for the Study of Tuberculosis of Bovine Origin prescribed for us. While in the midst of our studies with various culture media we were honored by the visit of Dr. Henry

C. Sweany, of Chicago, Illinois, who suggested a medium. The technique of preparation was given as follows:

Twenty grams of potato flour, 300 cc. of fresh milk and 100 cc. of fresh cream are measured out. The potato flour is placed in a sterile Erlenmeyer flask of 1500 cc., containing about 150 g. of glass beads; then the milk and the cream are added. The bottle is placed in boiling water for an hour; it is rotated until the mixture has the consistency of puree. It is then cooled to 60°C. and 450 cc. of whole eggs and 150 cc. of yolk are added. It is stirred well until a homogeneous emulsion is obtained and finally 20 cc. of a 2 per cent solution of malachite green are added. It is then filtered through sterile gauze and stirred with care until the mixture emulsifies uniformly. The mixture is then tubed and coagulated in inclined position for half an hour at 85°C.

This simple medium, of such easy preparation was tried out for the purpose of comparison with other media, the characteristics of which for the cultivation of the bovine bacillus are well known. Included in these is a special medium of Petragnani (9) in which the author replaced the glycerin with wax after having satisfied himself that wax is a substance which is favorable for the metabolism of the bacillus of Koch. The incorporation of wax in the above mentioned medium and the evaporation of chloroform in which the wax was dissolved, however, is an operation which we have never been able to effect in the ten minutes prescribed by the author. We have had to extend the time sometimes up to an hour.

We confirmed, however, what Petragnani states in his original work, namely, that this medium is not favorable for the maintenance of the transplantations of the bovine bacillus. Our comparative tests were made, therefore, with the media of Petragnani, Loewenstein, Sweany, and some variations of the latter.

With the exception of the culture medium of Loewenstein, the other media did not have in their composition asparagin or glycerin. We did not use other media which are recommended for the culture of the bovine bacillus, such as that of Kirchner (10) or Laporte (11), as they require for their preparation the serum of cattle or of horses, which not all laboratories have available in the necessary quantities.

The first series of sowing were made, using culture medium of Loewenstein, formula 1933, designated as (L) in table 2, the same without glycerin (X), the same with glycerin in a proportion of 0.75 per cent to the total volume (J), of Petragnani with wax (PC), of Sweany's original (S), of Sweany modification No. 1,[2] designated as W, and finally the medium of Sweany modification No. 2[3] designated as SP.

The materials sown were the omentum of the rabbit injected intraperitoneally

[2] Twelve yolks and three whole eggs, respectively, were used instead of 150 cc. yolks and 450 cc. whole eggs, respectively, with the 300 cc. of milk and 100 cc. of cream.

[3] The milk and cream were sterilized by means of live steam for one hour and the period of the inspissation was extended to an hour on three consecutive days. Also the 20 g. of potato flour was replaced by 80 cc. potato broth prepared in advance in accordance with our previous publication (6) wherein 500 g. of cubed potatoes were autoclaved for two hours in 1 liter of water containing 1 cc. sulphuric acid c.p. and the digest neutralized with normal sodium hydroxide to pH 7.

with pulp of bovine tuberculous lymph nodes, lung of rabbit inoculated intravenously with a 0.01 mg. of bovine culture originating from a child with Pott's disease, and the remaining 9 from bovine tuberculous lymph nodes.

The treatment of the above mentioned materials was the following: trituration in a mortar and addition of 3 cc. of a 4 per cent solution of sodium hydroxide, filling small tubes containing 0.5 cc. of a sterilized blue litmus and leaving it for twenty minutes in the incubator at 38°C. It was then neutralized with a 5 per cent solution of sulphuric acid and the same number of drops sowed on each tube of medium. In this experiment we did not filter the material through filter paper because on a previous occasion, when we did so, the development was very poor

TABLE 2

Number	L	X	J	P. C.	S	S. P.	W
1	—	cc	—	cc	ll	lll	cc
2	iii	ccc	iii	cc	ccc	llr	—
3	—	lll	cc	cc	lll	lll	ll
4	r	c	—	C	ll	lll	ccr
5	—	—	—	mm	l	l	c
6	—	l	c	cc	l	ll	c
7	—	c	—	cc	l	lll	ccc
8	—	infec.	ccc	c	l	lll	infec.
9	—	ccc	rrr	mmm	lll	lll	lll
10	—	cc	cc	mm	l	infec.	ll
11	—	c	ccc	ccc	cc	lll	lll

c—transparent flat colonies up to a diameter of 1 mm. with small central prominence.

l—white smooth semi-spheric colonies, up to a diameter of 1 mm.

i—colonies of the intermediate type, white, semi-spheric of granulated surface of 1 to 3 mm. diameter.

r—small rugose colonies up to a diameter of 1 mm.

m—colonies with or without a flat transparent base, of sinuous borders, prominent smooth centre or in the form of a mulberry, up to a diameter of 1 mm.

One letter means up to 20 colonies, two up to 50 and three more than 50.

and in many cases negative. Instead, we passed the suspension through a fine layer of sterile cotton.

These results correspond to readings made between thirty and forty-five days in the incubator at 38°C.

All the specimens were dysgonic with the exception of No. 2, which corresponded to a culture of bovine bacillus isolated from man, presenting a type of intermediate growth in the glycerinated medium and a dysgonic growth of small and flat colonies in those without glycerin. In the medium of Sweany, made with potato broth, there was generally a typical bovine type with a few rugose and flat colonies.

Second test: We used tuberculous material from guinea pigs inoculated with market cream (No. 1); first a rabbit inoculated with bovine bacilli obtained from man's gastric contents (No. 2); of sputum (No. 3); of bovine tuberculous lymph

nodes (Nos. 4, 5, 6 and 8); and of second growth of bovine bacilli which were obtained directly from bovine lymph nodes (Nos. 8, 9 and 10).

In this experiment the material was previously treated with a 5 per cent solution of hydrochloric acid for twenty minutes at 38°C. and then neutralized with 4 per cent sodium hydroxide. The tuberculous organs were filtered through a fine layer of cotton and the material from the cultures was filtered through filter paper.

The media tested were those of Loewenstein (L), the same without glycerin (X), the medium A which we described in a previous work (6), the same without glycerin (A sin), that of Sweany (S), the same with 80 cc. of potato broth instead of potato flour suggested by the author (SP), the medium of Sweany with addition of the salts corresponding to medium A, without glycerin (SA), that of Dorset (D), that of Petragnani with wax (PC) and that of Petragnani without peptone (Pc. sin p.).

TABLE 3

Number	L	X	A	A sin	D	P. C.	Pc. sin p.	S	S. P.	S. A.
1	ccc	lll	RR	mm	l	—	—	lcc	lcc	ccc
2	ccc	ccc	ccc	ccc	cc	c	cc	lll	lll	ccc
3	—	ccc	c	ccc	c	llc	ccc	lll	lll	llc
4	—	c	—	c	—	—	—	l	l	—
5	—	—	—	ll	c	—	—	lc	lc	ll
6	—	l	c	l	—	—	—	ll	ll	lc
7	—	cc	c	ccc	ccc	cc	c	l	ll	ll
8	—	ccc	c	cll	ccc	cc	ccc	lll	lll	lll
9	—	ccc	—	cll	ccc	cc	ccc	lll	lll	ccc
10	c	ccc	c	ccc	ccl	cc	cc	lll	lll	lll

For symbols, see table 2.

Of all these culture media two lots were made, one with malachite green and the other with congo red.

The readings were made between thirty and forty-five days of maintenance in the incubator at 38°C.

The signs have the same meanings as in the previous table, with the addition of R, which means rough colony, 1.5 mm. in diameter.

In the readings made after sixty days the only variation noted is that many of the small and semi-spheric colonies which correspond to dysgonic cultures in the media S. A., A sin, and X are represented as flat and thin which can clearly be seen with a lens; on other occasions, after some time, small colonies in form of a "mulberry" may appear as a central prominence or simply as another semi-spheric superimposed colony.

CONCLUSIONS

Glycerin in the proportion of 3 to 5 per cent in culture media seems to accentuate the dysgonism of the bovine tubercle bacillus and favor the eugonism of the

human type, the respective colonies of which manifest these morphological characteristics. On the contrary, the absence of glycerin in the medium of Loewenstein makes the human bacillus dysgonic. This dysgonism has been maintained for at least a year in the subsequent transplants made each month or every second month.

The glycerin which enters into the composition of the medium of Loewenstein (formula 1933) does not change the virulence of the first or second cultivation of the bacillus of Koch originating from man, irrespective of its being of human or bovine type.

The treatment of the material with 4 per cent sodium hydroxide or 5 per cent hydrochloric acid for twenty minutes at a temperature of 38°C. does not produce noteworthy differences with reference to the vitality of the bovine bacillus of Koch.

The morphology of the colonies of first cultivation depends greatly upon the composition of the culture medium.

There was a slight advantage in favor of congo red over malachite green as far as the number and size of the colonies were concerned.

The culture medium of Sweany with the addition of potato extract afforded the best means for the direct cultivation of the bacillus of Koch originating from either tuberculous lesions of cattle, or rabbits inoculated with bovine bacilli obtained from man. Subcultures from Sweany's medium develop into more luxuriant cultures and larger colonies than on the other medium used, and it also facilitates better the cultivation, preservation and typing of the specimens.

SUMMARY

By seedings made with sputa from tuberculous persons on the culture medium of Loewenstein and variations of the same, we find that the human strains in the media without glycerin are dysgonic.

This dysgonism is maintained in the subsequent transplants made each month or every second month for more than a year.

The virulence of the cultures so obtained is similar to that of cultures made with the same material in glycerinated culture media. This was proved by intrapleural injections of 0.01 mg. in the rabbit.

There are bacilli originating from man which are eugonic in the glycerinated media but which present the type of virulence of bovine strains for the rabbit.

The dysgonic bacilli with virulence corresponding to the bovine strains isolated from man become more quickly eugonic in the glycerinated media than those isolated from cattle.

As long as there is no culture medium which may permit the development at the same time of the human and bovine types of bacilli without imposing great alterations upon the characteristic morphology of the colonies, we recommend the use of two culture media separately for the typing of specimens originating from man, namely, one with glycerin in a proportion of not less than 3 per cent and one without glycerin.

Among the media without glycerin, that of Sweany, slightly modified, has

given the best results for the culture and preservation of the bovine strains of tubercle bacilli.

Absolute proof of the type of certain strains from man must rest on the reaction in the rabbit or on more refined serological studies.

SUMARIO

Por medio de siembras verificadas con esputos de tuberculosos en el medio de Loewenstein y variaciones del mismo, se descubrió que las cepas humanas en los medios sin glicerina resultan disgónicas.

Esa disgonía se mantiene en los trasplantes subsecuentes verificados cada mes o cada dos meses por espacio de más de un año.

La virulencia de los cultivos así obtenidos es semejante a la de los preparados con la misma sustancia en medios de cultivo glicerinados, según se demostró mediante inyecciones intrapleurales de 0.01 mg. en el conejo.

Hay bacilos procedentes del hombre que son eugónicos en los medios glicerinados, pero que acusan para el conejo más virulencia que las cepas bovinas.

Los bacilos disgónicos de virulencia correspondiente a la de las cepas bovinas aisladas del hombre se vuelven eugónicos en los medios glicerinados, más rápidamente que los aislados del ganado vacuno.

En tanto no haya ningún medio de cultivo que permita la proliferación simultánea de los tipos humanos y bovino del bacilo sin imponer mayores alteraciones en la típica morfología de las colonias, recomiéndase el empleo de dos medios de cultivo distintos para la clasificación de los ejemplares procedentes del hombre, a saber: uno con una proporción de no menos de 3 por ciento de glicerina y otro sin glicerina.

Entre los medios sin glicerina, el de Sweany, levemente modificado, ha dado los mejores resultados para el cultivo y conservación de las cepas bovinas.

La prueba absoluta del tipo de ciertas cepas humanas debe reposar en la reacción en el conejo o en estudios serológicos más delicados.

REFERENCES

(1) Griffith, A. S.: British Commission: Second Interim Report on Human and Animal Tuberculosis, 1907, part 1, p. 23.

(2) Valtis, J., and van Deinse, F.: Effets du viellissement sur les cultures de bacilles tuberculeux de type bovin, Compt. rend. Soc. de biol., 1936, *122*, 895.

(3) Arena, A. R., and Cetrangolo, A.: Transformacion de una cepe de bacilos de Koch, tipo bovino, Rev. Soc. argent. de biol., 1937, *13*, 270.

(4) Jensen, K. A.: Reinzüchtung und Typenbestimmung von Tuberkelbazillenstämmen, Zentralbl. f. Bakt. (Abt. 1), 1932, *125*, 222.

(5) Löwenstein, E.: Die Methodik der Reinkultur von Tuberkelbazillen aus dem Blute, Deutsche med. Wchnschr., 1930, *56*, 1010.

(6) Arena, A. R., and Cetrangolo, A.: Influenzia de la glicerina y asperagina sobre los cultivos del bacilo de Koch en medios nutritivos sólidos, An. Cáted. de pat. y clin. tuberc., 1941, *3*, 40.

(7) Löwenstein, E.: La Méthode d'hémoculture du virus tuberculeux et ses résultats, Ann. Inst. Pasteur, 1933, *50*, 161.

(8) Boquet, A., and Laporte, R.: Infection tuberculeuse expérimentale par voie pleurale, Compt. rend. Soc. de biol., 1935, *118*, 741.

(9) PETRAGNANI, F.: Terreno con cerz vergine per l'isolamento e la diagnosi del ceppo di tuberculosi bovina dal materiali tuberculari patologici, Boll. d. Ist. sieroterap. milanese, 1931, *10*, 554.

(10) KIRCHNER, M.: Experimentelles zur Prüfung der Frage inwieweit der Lupus auf humaner oder boviner Infektion beruht, Ztschr. f. Hyg. u. Infektionskr., 1922, *98*, 447.

(11) LAPORTE, R.: Etude microbiologique du bacille tuberculeux du type bovin, Ann. Inst. Pasteur, 1936, *57*, 400.

THE CUTANEOUS ACTIVITY OF OLD TUBERCULINS PREPARED BY THE SAME AND BY DIFFERENT METHODS[1]

R. Y. GOTTSCHALL, A. B. MITCHELL AND C. J. STRINGER

The method for standardizing tuberculin on sensitized guinea pigs devised by Lewis and Aronson (1) and Eagleton and Baxter (2) has been used in some laboratories but it has not gained universal acceptance. In a recent report Jensen *et al.* (3) found it impracticable to determine the potency of a purified tuberculin by the usual method of comparing the reactions produced by varying dilutions of a purified tuberculin on one side of the guinea pig with varying dilutions of the International Standard Old Tuberculin on the other. They point out that qualitative differences exist between tuberculins and for this reason suggest that there should possibly be two standards, a purified standard and an International Standard Old Tuberculin. Seibert and DuFour (4) consider the guinea pig inadequate as a means for determining the potency of tuberculins but suggest that it might be used preliminary to titration on human subjects. They mention a tuberculin tested by Glenn which appeared to have a potency ratio of 1:2 when tested on human subjects but which appeared to be equal in potency when tested on 6 guinea pigs. McCarter and Watson (5) also consider the guinea pig less sensitive than man as an indicator of tuberculin activity. However, Clark and Follin (6) suggest the guinea pig, in which sensitization is controlled, as a test animal for quantitative standardization of the purified protein derivative of tuberculin.

It was hoped that the test on sensitized guinea pigs as devised by Lewis and Aronson (1), Eagleton and Baxter (2), and Okell and Parish (7), and as modified by Bunney and Gottschall (8) for determining the potency of diluted tuberculin after storage or heating would also prove satisfactory for titrating Old Tuberculins. In this test, which was devised for testing heated or stored diluted tuberculin for loss in potency, a serial dilution of the freshly prepared tuberculin is compared with a serial dilution of the stored or heated diluted tuberculin. When this method was employed for the titration of tuberculins prepared by Dorset's method (9), using the International Standard for control, it became increasingly evident that the variation in titer between animals injected with the same dilutions made interpretation of the results difficult and the test of doubtful value. Comparison of reactions elicited by two tuberculins prepared by the same method generally showed much less variation in titer from animal to animal. We sought, therefore, to ascertain whether variations in titre among sensitive guinea pigs were due to methods of preparing the Old Tuberculin. The dilutions shown to be equivalent by guinea pig titrations were then tested on human subjects to determine whether the guinea pig could be relied upon as a test animal for titrating tuberculins prepared for human use.

[1] From the Bureau of Laboratories, Michigan Department of Health and the Ingham County Sanatorium, Lansing, Michigan.

PREPARATION OF TUBERCULINS

The culture used for preparing these tuberculins was the "Dt" strain obtained from the Bureau of Animal Industry.

Tuberculins 414, 461, 504 and 510 were prepared by the same method, while 405 and 411 were prepared by different methods. Details of the preparation of these products are as follows:

No. 414: This tuberculin was prepared according to Dorset's method (9), except that before the concentration procedure 100 ml. of glycerine for each original litre of culture medium was added. Filtrate and glycerine were then concentrated on the steam bath to one fifth the original volume of culture medium. The concentrate was preserved with 0.5 per cent phenol and filtered through a Mandler filter. The potency was determined on guinea pigs by titration with the International Standard Old Tuberculin. The dilution approximately equivalent to the Standard was 1:33,300. In this and other tuberculins the dilutions recorded are based on a 10 fold concentration of the filtrate calculated from the original volume of medium. A sufficient quantity of 50 per cent glycerine preserved with 0.5 per cent phenol was added to bring the entire volume to this titre and the product was refiltered. It was used for a control against which the other tuberculins were titrated. The pH of the pooled cultures at harvest was 8.1. During concentration the pH decreased to 6.0.

No. 405: Glutamic acid was substituted for asparagin in the Dorset medium formula. The pH of the medium was adjusted to 7.0 before sterilization. Nine weeks after inoculation the pellicles were water-logged and the pH of the cultures was 5.4. After steaming for three hours and straining out the organisms 50 ml. of glycerine was added for each original liter of culture medium and the glycerine and filtrate concentrated to one tenth the original volume of medium. It was preserved with 0.5 per cent phenol and filtered.

No. 411: Twenty-one week old cultures on Long's synthetic medium (10) were steamed, pooled and concentrated to one tenth the original volume of medium. The pH of the culture filtrate before concentration was 7.3.

No. 461: This preparation was made fifteen months after No. 414 but by the same procedure. The cultures were harvested nine weeks after inoculation. The pH before concentration was 7.6.

No. 504: The same method was used for preparing this lot of tuberculin as for preparing No. 414. It was made three years after 414. The age of the cultures at the time of harvest was ten weeks, the pH being 8.4.

No. 510: The preparation of this lot was similar to No. 414. It was prepared one month previous to 414. The cultures were harvested eight weeks after inoculation, the final pH being 8.3.

METHODS OF TITRATION

Three different methods of titrating tuberculin were employed in this study:

(*A*) The serial dilution method on sensitized guinea pigs as previously described (8). This consisted of a comparison of the reactions elicited by three series of serial dilutions:

(*a*) serial dilutions of a 1:33,300 dilution of the control (approximately equivalent to a 1:10,000 dilution of the International Standard); (*b*) serial dilutions of a 1:41,600 dilution of the control (80 per cent of the value of the control); and (*c*) serial dilutions of the unknown tuberculin. The potency of the latter series was evaluated by comparison with the two control series and recorded as being: (*1*) >1:33,300; (*2*) 1:33,300; (*3*) 1:37,000 when the unknown series was greater than the 1:41,600 control series but less than the 1:33,300 control series; (*4*) 1:41,600, when it was equivalent to the 1:41,600 control series; or (*5*) <1:41,600. The unknown was diluted, if necessary, until a dilution was obtained which appeared to be comparable in potency to the 1:33,300 control series.
(*B*) Comparison of the reactions elicited by single injections of a dilution of the control and unknown tuberculin. These injections were placed in exactly corresponding sites on the closely clipped backs of sensitized guinea pigs.
(*C*) Comparison of the equivalent values obtained by the above titrations on human subjects. These injections were made on corresponding sites of the flexor surface of the forearms 8 to 10 cm. from the wrist.

In the guinea pig titrations, readings were made twenty-four hours after injection, at which time the reactions were at their height. In the serial dilution method the three series of reactions were graded but were not measured, while the reactions elicited by two injections placed on exactly corresponding sites of the guinea pig's back were measured in two dimensions. Measurement was facilitated by marking the margins of the reactions with ink. In the tests on human subjects the readings were made in forty-eight hours, measurements likewise being taken in two dimensions. In guinea pigs and human subjects measurements included both the central area of infiltration and the surrounding erythema. Those guinea pigs or human subjects in which one or the other or both reactions failed to show definite margins were not included in the calculations. Our experience in conducting these tests has been that approximately 27 per cent of the human subjects and 87 per cent of the sensitized guinea pigs had well defined reactions with definite margins.

Syringes cleaned with chromic acid cleaning solution and needles boiled in soap solution as suggested by Nelsen *et al.* (11) were used in all tests. The diluent was unpreserved saline or borax-boric acid buffer. No preservative was used because Aronson (12) has shown that phenol sometimes gives reactions in tuberculous human subjects. All solutions were freshly prepared and in the guinea pig titrations the injections were made within an hour after dilution. The preparations used for testing on human subjects were also freshly prepared. They were never more than six hours old.

Guinea pigs sensitized by infection with the H37 strain of *M. tuberculosis* were used for titrating OT 405 and 461 by the serial dilution method. For all other titrations guinea pigs sensitized by injection of dead tubercle bacilli emulsified in liquid petrolatum according to the method of Freund and Gottschall (13) were used. A number of comparative tests indicated that identical results were obtained with these two methods of sensitization.

The potency in guinea pigs and human subjects in which two injections were placed on exactly opposite sides of the guinea pigs' backs or the arms in human subjects was determined by calculating the ratio of the average total areas and

by obtaining the mean and the median of the individual ratios. The standard deviation of the distribution was also obtained.

Titration of Tuberculins

The protocol showing the results when OT 405 was titrated with OT 414 by the serial dilution method is given in table 1. The data show a large deviation in titre among animals injected with the same dilutions. In the comparison of the 1:18,400 dilution, for instance, 3 animals indicated that it was weaker than a 1:41,600 dilution of 414, 2 animals showed that it was equivalent to a 1:41,600 dilution, in one it was equivalent to 1:37,000, in one to 1:33,300 while in 5 other animals a 1:18,200 dilution was stronger than a 1:33,300 dilution of 414. The dilution of 405 selected as being approximately equivalent to a 1:33,300 dilution of 414 was 1:20,000. Titration of this dilution by a comparison of single injections gave a ratio of 0.92 (table 2, experiment 1). On human subjects a ratio of

TABLE 1

Comparison of OT 405 with OT 414 on guinea pigs by the serial dilution method

DILUTIONS OF OT 405	REACTIONS TO DILUTIONS OF OT 405 COMPARED TO REACTIONS TO DILUTIONS OF OT 414					TOTAL NUMBER OF ANIMALS INJECTED	NUMBER OF ANIMALS WITH UNSATISFACTORY REACTIONS
	Dilutions of OT 414						
	1:41,600		1:37,000	1:33,300			
	Number of animals						
1:14,300					3 >	6	3
1:15,400					2 >	3	1
1:16,700	1 <		1 =	1 =	2 >	9	4
1:18,400	3 <	2 =	1 =	1 =	5 >	15	3
1:20,000	1 <	1 =	2 =	3 =		12	5
1:22,300	1 <		1 =	1 =		3	0

0.29 was obtained indicating 405 to be much weaker than 414 (table 3, experiment 1).

Comparison of a 1:6,600 dilution with a 1:33,300 dilution of 414 gave a ratio of 1.16, indicating that 405 was two to three times weaker by titration on human subjects than by titration on sensitized guinea pigs (table 3, experiment 2).

Since there was so much variation in titre among guinea pigs, and the human and guinea pig titres agree so poorly, purified protein derivatives of these two products were prepared according to the method of Seibert (14) to determine whether the responsible factors were eliminated by purification. The results of the tests of these two products by the serial dilution method are given in table 4 and again show a wide variation in potency from animal to animal, and from the data a value of 405 equivalent to 414 is not demonstrable. After the reactions on these animals had subsided, that is, after four to six weeks, the animals were again used for titration but the method employed was that of comparing single injections. Unfortunately some of the animals died in the interim. The data given in table 5 and table 2, experiment 2, indicate that a 1:5,700 dilution

of PPD 405 is equivalent to a 1:20,000 dilution of 414. The same animals were used in an attempt to determine whether those that gave larger or smaller reactions to PPD 405 than the majority of other animals in the group, when titra-

TABLE 2

Titration of Old Tuberculins and purified protein derivatives on guinea pigs by a comparison of single injections

EXPERIMENT NUMBER	TUBERCULINS AND DILUTIONS	AVERAGE SIZE REACTIONS IN MM.	RATIO OF TOTAL AREAS	MEAN RATIO	STANDARD DEVIATION	MEDIAN	NUMBER OF ANIMALS
1	OT 414, 1:33,300	16.7 x 19.5					
	OT 405, 1:20,000	15.9 x 18.9	0.92	0.94	0.17	0.90	20
2	PPD 414, 1:20,000	11.9 x 15.0					
	PPD 405, 1: 5,700	11.7 x 15.0	0.98	1.03	0.23	1.00	28
3	OT 414, 1:33,300	13.3 x 15.8					
	OT 411, 1:18,200	13.1 x 15.9	0.99	1.00	0.16	1.00	26
4	OT 414, 1:33,300	14.2 x 17.5					
	OT 461, 1:30,800	14.3 x 18.2	1.05	1.05	0.13	1.05	23
5	OT 414, 1:33,300	15.5 x 18.8					
	OT 504, 1:33,300	15.1 x 18.7	0.97	0.96	0.07	0.96	17
6	OT 414, 1:33,300	16.0 x 19.8					
	OT 510, 1:25,000	16.2 x 20.2	1.03	1.03	0.14	1.00	25
7	OT 27, 1:28,500	14.5 x 18.2					
	OT 28, 1:25,000	14.8 x 18.4	1.03	1.04	0.15	1.06	19
8	OT 27, 1:28,500	14.3 x 18.0					
	OT 29, 1:25,000	14.2 x 18.5	1.02	1.03	0.10	1.04	18
9	OT 414, 1:33,300	13.6 x 16.9					
	OT 414, 1:33,300	13.7 x 17.3	1.03	1.04	0.12	1.00	21
10	OT 414, 1:33,300	14.2 x 17.6					
	OT 414, 1:33,300	14.3 x 17.5	1.00	1.01	0.10	0.99	18
11	OT 414, 1:33,300	15.6 x 19.7					
	OT 414, 1:41,600	15.3 x 19.0	0.95	0.95	0.14	0.93	16
12	OT 414, 1:33,300	17.6 x 20.9					
	OT 414, 1:55,500	14.2 x 17.5	0.68	0.69	0.14	0.66	20

tions were made by the serial dilution method, were due to experimental error or to the ability of some guinea pigs to react differently to tuberculins prepared in a different manner. Nine of these animals shown in table 4 are listed in table 6 which gives the results obtained when various dilutions of PPD 405 were compared to a 1:20,000 dilution of PPD 414. The data show that in 7 of the 9

TABLE 3

Titration of Old Tuberculins and purified protein derivatives on human subjects

EXPERIMENT NUMBER	TUBERCULINS AND DILUTIONS	AVERAGE SIZE REACTIONS IN MM.	RATIO OF TOTAL AREAS	MEAN RATIO	STANDARD DEVIATION	MEDIAN	NUMBER OF SUBJECTS
1	OT 414, 1:33,300	22.6 x 28.0					
	OT 405, 1:20,000	12.9 x 14.3	0.29	0.41	0.32	0.27	37
2	OT 414, 1:33,300	17.7 x 21.0					
	OT 405, 1: 6,600	19.5 x 22.1	1.16	1.50	0.81	1.35	25
3	PPD 414, 1:20,000	20.3 x 26.1					
	PPD 405, 1: 5,700	16.6 x 20.3	0.64	0.71	0.34	0.59	24
4	OT 414, 1:33,300	23.0 x 29.0					
	OT 411, 1:18,200	18.0 x 21.0	0.57	0.66	0.38	0.49	23
5	OT 414, 1:33,300	19.5 x 22.7					
	OT 461, 1:30,800	18.8 x 21.6	0.91	1.01	0.33	1.02	20
6	OT 414, 1:33,300	18.7 x 24.7					
	OT 504, 1:33,300	19.1 x 24.2	1.00	1.19	0.73	1.10	18
7	OT 414, 1:33,300	16.5 x 19.4					
	OT 510, 1:25,000	18.1 x 21.8	1.23	1.50	3.21	1.19	21
8	OT 414, 1:33,300	21.2 x 27.4					
	OT 510, 1:25,000	26.4 x 34.9	1.59	2.23	1.67	1.51	29
9	OT 27, 1:28,500	17.1 x 20.6					
	OT 28, 1:25,000	20.6 x 26.4	1.54	1.85	0.86	1.58	30
10	OT 27, 1:28,500	22.1 x 26.7					
	OT 28, 1:25,000	23.6 x 28.9	1.16	1.53	0.91	1.27	28
11	OT 27, 1:28,500	13.6 x 16.6					
	OT 29, 1:25,000	16.3 x 19.6	1.42	1.71	0.84	1.44	20
12	OT 27, 1:28,500	18.8 x 22.6					
	OT 29, 1:25,000	24.0 x 29.3	1.66	1.74	0.75	1.39	19
13	OT 414, 1:33,300	17.9 x 20.0					
	OT 414, 1:33,300	18.8 x 20.7	1.09	1.09	0.37	1.03	23
14	OT 414, 1:33,300	19.0 x 24.7					
	OT 414, 1:41,600	18.7 x 24.0	0.96	1.06	0.51	0.99	28
15	OT 414, 1:33,300	21.2 x 25.0					
	OT 414, 1:55,500	14.7 x 16.5	0.46	0.50	0.21	0.47	27

animals these reactions were duplicated. These unusually large or small reactions therefore were not due to experimental error but to actual differences in the

reacting power of the animals to tuberculins prepared by different methods. Tests on human subjects gave a ratio of 0.64 (table 3, experiment 3).

Titration of OT 411 with 414 by the serial dilution method again indicated that variations were numerous enough to make interpretation of the results

TABLE 4

Comparison of PPD 405 with PPD 414 on sensitized guinea pigs by the serial diltion method

DILUTIONS OF PPD 405	REACTIONS TO DILUTIONS OF PPD 405 COMPARED TO REACTIONS TO DILUTIONS OF PPD 414					TOTAL NUMBER OF ANIMALS INJECTED	NUMBER OF ANIMALS WITH UNSATISFACTORY REACTIONS
	Dilutions of PPD 414						
	1:41,600		1:37,000	1:33,300			
	Number of animals						
1:4,000					J712 >	3	2
1:5,000	C744* <		M219 =		(G727) (G722) > (U394)	6	1
1:5,300		C872 =		G768 =		3	1
1:5,700	(O684) (J508) < (G756)	J712 =	O671 =		(U658) > (G734)	7	0
1:6,600			N765 =		(F478) > (N720)	3	0
1:8,000	G725 <	(N1) (C671) = (T291)		(O363) (F479) =	125 >	8	1

* Guinea pig numbers.

TABLE 5

Titration of various dilutions of PPD 405 with a 1:20,000 dilution of PPD 414 on sensitized guinea pigs by the single injection method

DILUTIONS OF PPD 405	NUMBER OF ANIMALS	RATIO OF TOTAL AREAS
1:5,300	13	1.13
1:5,700	28	0.98
1:6,600	36	0.92
1:8,000	8	0.67

difficult (table 7). It is apparent, however, that a titre of 1:15,400 is approximately equivalent to a 1:33,300 dilution of 414. When titrated by a comparison of single reactions, the value as determined on 26 guinea pigs indicated that a 1:18,200 dilution was comparable to a 1:33,300 dilution of OT 414 (table 8 and table 2, experiment 3). The tests on sensitive human subjects gave a ratio of 0.57 (table 3, experiment 4).

The results of the titration of tuberculin 461 with 414 by the serial dilution method are given in table 9 and show that the titre comparable to a 1:33,300

TABLE 6

Reactions elicited by various dilutions of PPD 405 by the single injection method when compared to a 1:20,000 dilution of PPD 414 in those animals giving unusually large or small reactions by the serial dilution method

DILUTIONS OF PPD 405	COMPARATIVE REACTIONS ELICITED BY PPD 414 AND PPD 405								
	Guinea pigs								
	O684	G756	G725	C872	G734	F478	N720	N479	C744
1:5,000	<		<			>	>	>	≪ >
1:5,300	<		<	≪		>	>	>	>
1:5,700	≪*=	≪ <	<	<	≫ >	>	>	>	= >
1:6,600	=	<	< <	<	>	≫ >	≫ >	= =	= >
1:8,000			≪ <					= =	<

* Double symbols indicate original titration by the serial dilution method; single symbols indicate subsequent titrations by a comparison of single injections.

TABLE 7

Comparison of OT 411 with OT 414 on sensitized guinea pigs by the serial dilution method

DILUTIONS OF OT 411	REACTIONS TO DILUTIONS OF OT 411 COMPARED TO REACTIONS TO DILUTIONS OF OT 414					TOTAL NUMBER OF ANIMALS INJECTED	NUMBER OF ANIMALS WITH UNSATISFACTORY REACTIONS
	Dilutions of OT 414						
	1:41,600		1:37,000	1:33,300			
	Number of animals						
1:10,000					2 >	3	1
1:11,700				1 =	1 >	3	1
1:13,300	1 <		1 =	4 =	4 >	12	2
1:14,300		1 =			1 >	3	1
1:15,400	2 <	1 =	2 =	3 =	4 >	15	3
1:16,700	3 <					3	0
1:18,200	1 <	1 =	1 =	1 =		6	2
1:25,000	2 <					3	1

TABLE 8

Comparison of OT 411 with OT 414 on sensitized guinea pigs by the single injection method

DILUTIONS OF OT 411	NUMBER OF ANIMALS	RATIO OF TOTAL AREAS
1:13,300	13	1.2
1:15,400	29	1.1
1:18,200	26	0.99
1:22,200	9	0.97

dilution of 414 is 1:30,800. The protocol also shows that with this product the endpoint is much sharper with little deviation in titre among the animals injected with the same dilutions. A comparison of single injections (table 10 and

table 2, experiment 4) also indicates that a 1:30,800 dilution is equivalent to the control. The human studies likewise show that these two dilutions are approximately comparable giving a ratio of 0.91 (table 3, experiment 5).

Two other tuberculins, 504 and 510, which were prepared in the same manner as 461 and 414, were then titrated against 414. The results given in table 2, experiments 5 and 6, and table 3, experiments 6, 7 and 8, show that 504 and 414 are equivalent in both guinea pigs and human subjects. In OT 510, however, the titres obtained in the human titration do not agree with the titre obtained in the guinea pig. Neither of the ratios in the human titrations are significant

TABLE 9

Comparison of OT 461 with OT 414 on sensitized guinea pigs by the serial dilution method

DILUTIONS OF OT 461	REACTIONS TO DILUTIONS OF OT 461 COMPARED TO REACTIONS TO DILUTIONS OF OT 414					TOTAL NUMBER OF ANIMALS INJECTED	NUMBER OF ANIMALS WITH UNSATISFACTORY REACTIONS
	Dilutions of OT 414						
	1:41,600		1:37,000	1:33,300			
	Number of animals						
1:20,000					1 >	2	1
1:25,000					3 >	3	0
1:26,600				2 =		3	1
1:28,600					2 >	3	1
1:30,800				10 =	1 >	13	2
1:33,300			3 =	3 =		8	2
1:36,400	3 <					3	0
1:40,000	4 <	1 =	1 =			6	0
1:50,000	2 <					3	1

TABLE 10

Comparison of OT 461 with OT 414 on sensitized guinea pigs by the single injection method

DILUTIONS OF OT 461	NUMBER OF ANIMALS	RATIO OF TOTAL AREAS
1:26,600	23	1.11
1:30,800	23	1.05
1:36,400	10	0.81

but they seem to indicate that 510 is stronger than 414. The serial dilution method of titration on guinea pigs was not used on these two tuberculins.

Two other lots made in the same manner were also titrated. They were made from the three B.A.I. strains, Pn, Dt and C. Equivalent quantities of OT prepared from each strain were mixed in the preparation of these three products, Nos. 27, 28 and 29, otherwise the preparation was the same as for OT 414, 461, 504 and 510. The results of the titrations on guinea pigs are given in table 2, experiments 7 and 8. Each of these tuberculins was titrated twice on human subjects (table 3, experiments 9, 10, 11 and 12), and the tests indicate that 28 and 29 are somewhat stronger than 27.

As a final test to determine whether finer differences could be detected by titra-

tion on guinea pigs than by titration on human subjects a 1:33,300 dilution, a 1:41,600 dilution and a 1:55,500 dilution of OT 414 were titrated against a 1:33,300 dilution of the same tuberculin. In these titrations, reactions elicited by single injections were compared. The results given in table 2, experiments 9, 10, 11 and 12, and table 3, experiments 13, 14 and 15, show that detection of a 20 per cent difference in potency in guinea pigs and human subjects is questionable while a 40 per cent difference is readily detectable.

STATISTICAL ANALYSIS

An attempt has been made to compare the relative merits of guinea pigs and human subjects as test animals for determining the strength of tuberculins prepared by similar and by dissimilar methods. In the guinea pig we have a test animal artificially highly sensitized to tuberculin and closely controlled as far as other variables are concerned; in the human, a test animal of varying degrees of sensitivity and unknown pedigree. The human subjects used for these tests were a disinterested group of institutionalized, presumably well, people.

The preparation of the dilutions of the tuberculins and of the equipment was the responsibility of a single individual. The data pertaining to the guinea pig and that pertaining to the human, however, were collected by two persons working independently. It is not felt that this latter consideration has in any way affected the reliability of the data.

A tabulation of the data obtained in the use of guinea pigs appears in table 2 and that obtained in the humans in table 3. These tables show the tuberculins in the dilutions used for testing, the average size of the reactions observed in mm., the ratios of the total areas of the reactions, the mean and median of the individual ratios and standard deviation of their distribution together with the number of subjects on which readings were obtained. The comparison of the guinea pig and human as test animals is based on a comparison of the ratios obtained with corresponding dilutions of tuberculins. A comparison of the deviations of the individual ratios about their means was made to obtain some idea of the reliability of these ratios. The standard deviations of these distributions provide measures of the deviations as well as measures of significance of the mean ratios. The individual ratios were obtained for each subject by dividing the area of the reaction observed with the use of the tuberculin tested by that observed with the use of the control tuberculin. An important consideration not included in the tables is the actual variation in the individual ratios. A greater variation was observed in the reactions in both guinea pigs and humans to dissimilar tuberculins than to tuberculins prepared in a similar manner. This was more pronounced in humans than in guinea pigs.

With tuberculins made in a similar manner, in humans, the individual ratios were within the limits of chance variation in all but one or two instances in each group of individuals tested. These one or two widely divergent ratios account for the large standard deviations observed for all of these tuberculins except 510. The median ratio and the ratio computed from the total areas of the reactions were less affected by these widely divergent individual ratios than the mean ratio

and, for humans, may be considered better indices of the strength of a tuberculin. While these widely divergent ratios were felt to be the result of errors in conducting the tests on humans rather than the result of differences in response to the tuberculins used, no satisfactory reason for their exclusion could be established. Had they been excluded, the mean ratios obtained with similar tuberculins would have been significant ratios for all of these tuberculins except 510. Although two groups of individuals were tested with 510, a significant ratio was not obtained. In the comparison of three different dilutions of 414 in which a standard dilution of 414 was used as a control, in humans, the mean ratios were all significant ratios.

The individual ratios obtained in the guinea pig were less variable and correspondingly more reliable. All of the mean ratios obtained with the guinea pig were significant ratios and subject to a small standard error. These mean ratios closely approximated the median ratios and the ratios obtained from the total areas in each group of animals tested. The guinea pig, therefore, was a much better test animal than the human for testing tuberculins. Because of the marked variation observed in the individual ratios obtained in human subjects, approximately 100 persons with measurable positive reactions would be required to obtain as reliable ratios as were obtained with 25 guinea pigs.

While too few humans were included in each group tested for entirely satisfactory results, the data presented are adequate to justify the conclusions drawn from the study that tuberculins prepared by dissimilar methods give widely differing ratios in human subjects when compared to those obtained with guinea pigs, while closer agreement may be anticipated between the ratios obtained with these two animals with tuberculins made in a similar manner.

DISCUSSION

The foregoing experiments show that, when tuberculins prepared by different methods are titrated on sensitized guinea pigs, there is a greater deviation in titre from animal to animal than if the tuberculins are prepared by identical methods. In these experiments the standard deviations obtained with the two tuberculins prepared by different methods were 0.16 and 0.17, while those obtained with tuberculins prepared by identical methods varied from 0.07 to 0.15. Because of the variation in titre among animals injected with the same dilutions, the serial dilution method of titrating any tuberculin is not always satisfactory. Wu, Ten Broeck and Li (15) have shown that acid albumins and alkali albumins were immunologically different from the undenatured albumins from which they were prepared and that albumins denatured by different methods were closely related although not identical. It is plausible that tuberculins prepared in different ways would likewise be somewhat different and this might account for the deviation in titre among animals injected with the same dilutions.

Our results indicate that the human titre and the guinea pig titre disagree when tuberculins are prepared by methods differing from the control. Since the methods for preparing tuberculin are numerous (16), and wide differences exist between the human and guinea pig titre when tuberculins prepared by different methods are titrated, it would seem that the guinea pig is unsuitable as a test

animal for determining the potency of such tuberculins for human use. The guinea pig may be used for testing tuberculins made by methods similar to the control or products in which good correlation between the guinea pig and human titres are demonstrable.

Certain distinct differences exist between the reactions elicited by tuberculin in guinea pigs and in human subjects. The height of the reaction in guinea pigs is reached in twenty-four hours, while in the human the reactions are usually at their height in forty-eight hours. In the human there is a well defined zone of erythema surrounding the central indurated area, while this erythematous area is usually very small or absent in the guinea pig. These differences may be partially responsible for the different ratios obtained with tuberculins titrated on human subjects as compared with tuberculins titrated on guinea pigs.

Although our data give no indication as to whether the guinea pig titre or the human titre is correct, it would seem that the potency of different lots of tuberculin prepared by identical methods in the same laboratory should be approximately the same. This is verified by our guinea pig protocols but not by the human protocols.

SUMMARY

Tuberculins prepared by different methods, injected into sensitized guinea pigs, show a greater variation in the size of the reactions from animal to animal than do tuberculins prepared by similar methods. For this reason the serial dilution method of titration is not satisfactory for titrating tuberculins prepared by dissimilar methods. Variations also occur in sensitive human subjects injected with single dilutions of tuberculins prepared by different methods.

The titres obtained on human subjects do not always agree with those obtained on sensitized guinea pigs. The greatest deviation between the guinea pig and human titre occurs in those tuberculins prepared by different methods.

Statistical analysis supports the conclusion that the potency of tuberculins prepared in a similar manner is determined more reliably on guinea pigs than on human subjects.

SUMARIO

Las tuberculinas preparadas con diferentes técnicas, al ser inyectadas en cobayos sensibilizados, revelan mayor variación en la intensidad de las reacciones de un animal a otro que las preparadas con técnicas semejantes, por cuya razón la técnica de titulación a base de diluciones seriadas no resulta satisfactoria para titular tuberculinas preparadas conforme a técnicas diferentes. También se observan variaciones en los sujetos humanos sensibles a los que se les inyectan diluciones aisladas de tuberculinas preparadas con diversas técnicas.

Los títulos obtenidos en los sujetos humanos no convienen siempre con las obtenidas en cobayos sensibilizados, correspondiendo la desviación máxima en el título entre el cobayo y el hombre, a las tuberculinas preparadas con distintas técnicas.

El análisis estadístico apoya la conclusión de que la potencia de las tuber-

culinas preparadas en forma semejante, es determinada en forma más fidedigna en los cobayos que en los sujetos humanos.

We are grateful to Dr. Jules Freund and to Dr. H. C. Batson for their suggestions in the preparation of the manuscript.

REFERENCES

(1) Lewis, P. A., and Aronson, J. D.: Am. Rev. Tuberc., 1923, *7*, 404.
(2) Eagleton, A. J., and Baxter, E. M.: Brit. J. Exper. Path., 1923, *4*, 289.
(3) Jensen, J. A., Bindslev, G., Moller, S., Hansen, A., and Lind, P.: Tubercle, 1938, *19*, 386 and 433.
(4) Seibert, F. B., and DuFour, E. H.: Am. Rev. Tuberc., 1940, *41*, 57.
(5) McCarter, J. R., and Watson, D. W.: J. Immunol., 1942, *43*, 85.
(6) Clark, L. T., and Follin, S. F.: Am. Rev. Tuberc., 1942, *46*, 77.
(7) Okell, C. C., and Parish, H. J.: Brit. J. Exper. Path., 1927, *8*, 170.
(8) Bunney, W. E., and Gottschall, R. Y.: Proc. Soc. Exper. Biol. & Med., 1938, *39*, 71.
(9) Dorset, M.: J. Am. Vet. M. A., 1934, *103*, 154.
(10) Long, E. R., and Seibert, F. B.: Am. Rev. Tuberc., 1926, *13*, 393.
(11) Nelsen, W. E., Seibert, F. B., and Long, E. R.: J. A. M. A., 1937, *108*, 2179.
(12) Aronson, J. D.: Personal communication.
(13) Freund, J., and Gottschall, R. Y.: Arch. Path., 1942, *34*, 73.
(14) Seibert, F. B., Aronson, J. D., Reichel, J., Clark, L. T., and Long, E. R.: Supplement to Am. Rev. Tuberc., December, 1934.
(15) Wu, H., Ten Broeck, C., and Li, Chien-Pien: Proc. Soc. Exper. Biol. & Med., 1926, *24*, 472.
(16) Prausnitz, C.: Bull. Health Organ., League of Nations, Geneva, 1929, 52.

FURTHER OBSERVATIONS ON THE PRODUCTION OF AUTOLYTIC TUBERCULIN[1]

H. J. CORPER AND MAURICE L. COHN

In a previous study (1) it was noted that tubercle bacilli, grown on a simple nonprotein synthetic medium, removed and washed free from extraneous materials, autolyzed at incubator temperature (37°C.) at pH 7.0 to pH 8.0 to produce a maximum and biologically highly potent tuberculin (tuberculoprotein) within one week. The autolysis of human tubercle bacilli proceeded best at incubator temperature and was practically completely retarded at refrigerator temperature (3°C.). The autolysate obtained from human tubercle bacilli is biologically identical in activity to the tuberculin (tuberculoprotein) obtained in the natural filtrate from growing these bacilli on a nonprotein synthetic medium for over three months. This autolysate is free from contamination with extraneous constituents of the medium as well as foreign materials resulting from the growth of the bacilli. The tests at that time were performed in the usual manner of autolytic tissue tests. The open system with ready air access was used, since most of the autolytic reagents were only slightly volatile at incubator temperature and a satisfactory concentration in the liquid containing the washed tubercle bacilli could be maintained for the duration of the experiment. Two of the chemicals used, diethyl ether (B. P. 34–35°C.) and petroleum ether (B. P. 30–60°C.), were in the highly volatile class. Adequate saturation of the liquid containing the tubercle bacilli could not be maintained satisfactorily and, although they produced sufficient effect on the bacilli to consummate a liberation of tuberculin within the month period of the test, they did not destroy all the bacilli. However, such effect obtained in autolysis was not coincident with the loss of viability of the bacilli and might therefore lead to erroneous deductions regarding the action of the chemicals in the liberation of tuberculin. The question might be asked whether the liberation of tuberculin was a vital process or whether it occurred by enzymatic action after loss of viability of the cells. The growth limit tests with good media for testing viability of tubercle bacilli are too delicate to discern relative viability or more than complete loss of viability since less than 0.000,000,01 mg. of viable tubercle bacilli will give a positive culture (2). In order to maintain a constant concentration of the volatile chemical in the liquid containing the tubercle bacilli, the production of tuberculin and determination of viability were studied with the bacilli in suspension. An adequate amount of chemical was added and placed in glass sealed tubes.

In order to note differences between the open-to-air system of autolysis of tubercle bacilli and the closed tube (sealed in glass) system, an experiment was performed with pure toluene. A weighed amount (about 1 g. moist bacillary mass) of washed tubercle bacilli from a one month old growth, at 37°C., of human tubercle bacilli on a nonprotein synthetic medium (Wong-Weinzirl) was

[1] From the Research Department, National Jewish Hospital, Denver, Colorado.

placed in bacteriological tubes containing 10 cc. sterile distilled water and 5 mg. of sodium carbonate. The tubes were drawn down to small calibre in a flame and 0.5 cc. pure toluene was added, after which the tube was carefully sealed in the flame. These tubes were then placed in the incubator, shaken at intervals, and at regular periods (noted in table 1) a tube was removed to determine viability of the bacilli and tuberculoprotein content of the centrifugated and filtered liquid. The results are recorded in table 1.

The data in table 1 indicate that tubercle bacilli are killed by toluene in sealed tubes within four hours (the shortest interval tried) as compared with from one to two days when the toluene is open to air. Thus it appears that the phenomenon of toluene autolysis of tubercle bacilli with the consequent libera-

TABLE 1

Toluene autolysis of human tubercle bacilli in sealed tubes

TIME OF INCUBATION	MILLIGRAMS OF TRICHLORACETIC ACID PRECIPITABLE MATERIAL IN CENTRIFUGATED LIQUID	MILLIGRAMS OF TRICHLORACETIC ACID PRECIPITABLE PROTEIN IN FILTRATE AFTER FILTRATION THROUGH 1.5 PER CENT GUNCOTTON MEMBRANE
4 hrs.**	2.3*	1.6*
8 hrs.	2.4	1.4
12 hrs.	2.6	1.4
16 hrs.	2.6	1.5
20 hrs.	4.2	1.6
1 day	3.8	1.6
2 days	5.0	2.6
3 days	4.4	3.8
5 days	5.5	4.7
7 days	8.1	5.4

* Calculated by means of the Seibert factor from readings in Hopkins vaccine tubes for 1 g. of moist bacilli (containing 78.8 per cent water as determined by vacuum drying over phosphoric anhydride).

** In culture tests on a good nutrient medium, the tubercle bacilli were found to be nonviable at the four hour interval.

tion of tuberculin (tuberculoprotein) follows the death of the bacilli. No noteworthy differences in the liberation of tuberculoprotein was noted between the open-to-air or sealed-in-glass systems however. This is comprehensible on the basis of the rapid killing of the bacilli in either case by the toluene water.

The effect of diethyl ether (B. P. 34–35°) on the viability of the tubercle bacilli and the liberation of tuberculoprotein by autolysis in sealed glass tubes was studied in the same way that toluene was tried and following the same technique as that used for toluene and described above. The results of this study are recorded in table 2.

The results recorded in table 2 indicate that the diethyl ether does not kill all the tubercle bacilli until after the third day of incubation at 37°C. in a glass sealed tube. However, the results of the graded plantings would indicate that the majority have not survived beyond the first day of incubation. This experi-

ment makes it comprehensible that some of the bacilli survived up to one month in the earlier open system method because contact with the highly volatile diethyl ether was not sufficiently and adequately maintained. Since many of the bacilli had been destroyed by the diethyl ether even in the open system, a fairly good autolysis of the bacilli occurred and a good liberation of tuberculoprotein resulted.

TABLE 2

Diethyl ether autolysis of human tubercle bacilli in sealed tubes*

TIME INCUBATED	MILLIGRAMS OF TRICHLORACETIC ACID PRECIPITABLE MATERIAL IN CENTRIFUGATED LIQUID	MILLIGRAMS OF TRICHLORACETIC ACID PRECIPITABLE PROTEIN IN FILTRATE AFTER FILTRATION THROUGH 1.5 PER CENT GUNCOTTON MEMBRANE	GROWTH OF BACILLI FROM MILLIGRAMS PLANTED			
			1.0	10^{-2}	10^{-4}	10^{-6}
1 day	2.4**	2.2**	+	+	+	0
2 days	4.4	2.5	+	0	0	0
3 days	8.1	5.3	+	0	0	0
5 days	9.5	5.8	0	0	0	0
1 week	11.4	6.1	0	0	0	0

* B. P. 34–35°C. at 760 mm.

** Calculated by means of the Seibert factor from Hopkins tubes readings for 1 g. of moist bacilli (containing 78.8 per cent water).

TABLE 3

Petroleum ether autolysis of human tubercle bacilli in sealed tubes*

TIME INCUBATED	MILLIGRAMS OF TRICHLORACETIC ACID PRECIPITABLE MATERIAL IN CENTRIFUGATED LIQUID	MILLIGRAMS OF TRICHLORACETIC ACID PRECIPITABLE PROTEIN IN FILTRATE AFTER FILTRATION THROUGH 1.5 PER CENT GUNCOTTON MEMBRANE	GROWTH LIMIT IN MILLIGRAMS PLANTED			
			1.0	10^{-2}	10^{-4}	10^{-6}
1 day	1.3**	1.0**	+	+	0	0
2 days	6.7	2.4	+	+	0	0
3 days	6.7	3.9	+	0	0	0
5 days	6.7	4.6	0	0	0	0
1 week	7.4	5.4	0	0	0	0

* B. P. 30–60°C. at 760 mm.

** Calculated by means of the Seibert factor from Hopkins tubes readings for 1 g. of moist bacilli (containing 78.8 per cent water).

Petroleum ether (B. P. 30–60°C.), like diethyl ether, was found not to kill all the tubercle bacilli within one month when used in an open-to-air system because of its high volatility. It did permit autolysis of the bacilli, however, and was tried by the closed system (sealed in glass tube) technique with the results recorded in table 3.

The findings with petroleum ether used to autolyze tubercle bacilli in the closed system at incubator temperature indicate that this reagent will kill all the bacilli only after three days while many of them are destroyed within the

first and second days of exposure. In the closed system, the liberation of tuberculin is very similar to that in the open system but probably more efficient, since the bacilli are all destroyed within about three days. In the open system, some of them may remain alive for as long as one month either because of inadequate contact with the petroleum ether or because of inadequate concentration maintained throughout the experiment.

SUMMARY

In a closed system (sealed in glass tubes), diethyl ether and petroleum ether with low volatilization temperatures destroy washed viable tubercle bacilli suspended in watery solution within about three days at incubator temperature (37°C.) and permit autolysis of these bacilli with liberation of tuberculoprotein within about one week. This is in contrast to the effect in an open-to-air system when the viability of all the bacilli is not destroyed within one month because of the volatility of the reagent and inadequate concentration contact with the bacilli.

SUMARIO

En un dispositivo cerrado (cierre a la llama en tubos de vidrio) el éter dietílico y el éter de petróleo de bajos puntos de volatilización destruyen los bacilos tuberculosos viables lavados y suspendidos en solución acuosa, en término de unos 3 días a la temperatura de la estufa (37°C.) y permiten la autólisis de esos bacilos con desprendimiento de tuberculo-proteína, aproximadamente en término de una semana. Este resultado hállase en contraposición al efecto observado en un dispositivo expuesto al aire en el cual la viabilidad de todos los bacilos no es destruída en término de un mes debido a la volatilidad del reactivo y a ser inadecuada la concentración de éste, que se pone en contacto con aquéllos.

REFERENCES

(1) Corper, H. J., and Cohn, Maurice L.: Autolysis of tubercle bacilli and the production of tuberculin (tuberculoprotein), Am. Rev. Tuberc., 1943, *48*, 443.

(2) Corper, H. J., and Cohn, Maurice L.: Media for tubercle bacilli: An evaluation of different media for diagnostic cultures of tubercle bacilli, Am. Rev. Tuberc., 1942, *46*, 560.